Acta Physiologica

Acta Physiol 2010, **198**, 203–208

REVIEW

A decade of hypocretins: past, present and future of the neurobiology of arousal

L. de Lecea

Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, CA, USA

Received 25 March 2009, revision requested 21 April 2009, revision received 21 April 2009, accepted 22 April 2009
Correspondence: L. de Lecea, Department of Psychiatry and Behavioral Sciences, Stanford University, 701 B Welch Rd, Palo Alto, CA 94304, USA.
E-mail: llecea@stanford.edu

Abstract

In 1998, two groups independently identified the hypocretins, also known as orexins, as two hypothalamic peptides derived from the same precursor expressed in a few thousand neurones restricted to the perifornical area. A decade later, an amazing set of discoveries has demonstrated a key role for this neurotransmitter system in arousal and beyond. Here I review some of the experiments that led to these discoveries and the implications in the neurobiology of the hypothalamus and our understanding of brain arousal.
Keywords addiction, hypothalamus, optogenetics, orexin, sleep, wakefulness.

Discovery of the hypocretins

To illuminate additional molecules that contribute to the specialized functions of hypothalamic nuclei, we embarked on a systematic analysis of the mRNAs whose expression is restricted to or enriched in the hypothalamus. We used directional tag PCR subtractive hybridization (Usui *et al.* 1994) to enrich a cDNA library for clones of mRNA species selectively expressed in the hypothalamus. We studied a sample of 100 clones selected because of their enrichment in the subtracted library (Gautvik *et al.* 1996). These clones corresponded to 43 distinct mRNA species, about half of which were novel. Thirty-eight of these 43 mRNAs (corresponding to 85 of the clones in the sample) exhibited enrichment in the hypothalamus; 23 were highly enriched. As many as 40% of the hypothalamus-enriched sequences encoded secreted proteins, suggesting that, in contrast to other brain regions such as the striatum, the hypothalamus specializes in producing intercellular signalling molecules. Among the clones showing the highest degree of hypothalamus enrichment were cDNAs for oxytocin, vasopressin, CART, melanin concentrating hormone, POMC, VAT-1 and a novel species called clone 35 (de Lecea & Sutcliffe 2005).

In situ hybridization on coronal sections of brain from adult male rats was performed, using the inserts from clones representing many of the RNAs, including clone 35. The clone 35 mRNA displayed a striking pattern of bilaterally symmetric expression restricted to a few cells in the perifornical region of the lateral hypothalamic area (Fig. 1), with no signals outside the hypothalamus (Gautvik *et al.* 1996).

The 569-nucleotide rat sequence (de Lecea *et al.* 1998) suggested that the corresponding mRNA encoded a 130-residue putative secretory protein with four pairs of tandem basic residues for potential proteolytic processing. The absence of this site and the nature of the other differences made it unlikely that two of the four possible rat maturation products were functional. The two remaining putative peptides were absolutely preserved between rat and mouse. Both of these terminated with glycine residues, which in proteolytically processed secretory peptides typically are substrates for peptidylglycine alpha-amidating monooxygenase, leaving a C-terminal amide in the mature peptide. These features suggest that the product of the clone 35 hypothalamic mRNA served as a pre-prohormone for two C-terminally amidated, secreted peptides. One of these, which was later to be named hypocretin 2 (hcrt2), was, on the basis of the putative pre-prohormone amino acid sequence, predicted to contain precisely 28 residues. The other (hcrt1) had a defined predicted amidated C terminus but, because of uncertainties as to how the amino terminus might be proteolytically processed, an undefined N-terminal extent.

 doi: 10.1111/j.1748-1716.2009.02004.x

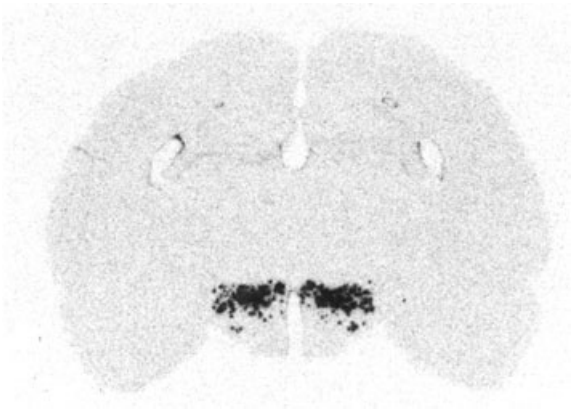

Figure 1 The first autoradiograph showing Hcrt mRNA distribution in the lateral hypothalamus.

Sakurai and colleagues described the Hcrt peptides, which they called orexins, as ligands of two orphan G-protein coupled receptors and demonstrated that intracerebroventricular (icv) administration of either hcrt1 or hcrt2 increased food consumption in rats (Sakurai *et al.* 1998). Furthermore, rats fasted for 48 h increased the concentration of hypocretin mRNA and peptides. The N-terminus of orexin A (hcrt1) was defined and found to correspond to a genetically encoded glutamine derivatized as pyroglutamate. The two intrachain disulphide bonds within hcrt1 were also defined. Orexin B is identical to hcrt2. These two parallel discoveries initiated a journey of discoveries that have redefined our knowledge of the neurobiology of sleep and wakefulness (Table 1).

Outputs and inputs

One of the most distinctive features of the hypocretinergic system is its restricted localization to a few thousand neurones in the lateral hypothalamus. We used an antiserum against bacterially expressed preproHcrt to map the projections of these few thousand neurones in the rat brain (de Lecea *et al.* 1998). The highest density of Hcrt-immunoreactive fibres is observed within the hypothalamus, with innervations in the lateral, dorsal and posterior areas, the paraventricular hypothalamic nucleus, arcurate nucleus and supramammillary nucleus (Peyron *et al.* 1998, Date *et al.* 1999, Nambu *et al.* 1999). Outside the hypothalamus, high Hcrt terminal density can be observed in the following brain nuclei: locus coeruleus (LC), septum, bed nucleus of the stria terminalis, thalamic paraventricular and reunion nuclei, periaqueductual grey, substantia nigra, raphe peribrachial pontine region, medullary reticular formation and the nucleus of the solitary tract. Less prominent projections are detectable in the cortex, amygdala, hippocampus and olfactory bulb (Peyron *et al.* 1998, Chen *et al.* 1999, Date *et al.* 1999). One of the primary reciprocal projection bundles innervates nuclei of the ascending arousal system such as the cholinergic pedunculopontine and laterodorsal tegmental nuclei, the serotonergic dorsal and median raphe nuclei, the noradrenergic LC, and the histaminergic tuberomammillary nucleus (Peyron *et al.* 1998). These set of target regions, most of which are associated with arousal, led us to speculate that activation of Hcrt neurones results in increased wakefulness (Peyron *et al.* 1998).

By using conventional (Yoshida *et al.* 2006) and transgene-based (Sakurai *et al.* 2005) retrograde tracing several groups have shown that hypothalamic nuclei involved in circadian and metabolic integration such as the arcuate nucleus, dorsomedial hypothalamus (DMH) and paraventricular nuclei send projections to Hcrt cells. Among other structures that send descending projections to Hcrt neurones are the limbic system, the forebrain, subcortical and thalamic nuclei. Circadian modulation of Hcrt cells is suggested by the presence of

Table 1 Chronology of milestone discoveries related to the hypocretinergic system

1998	Molecular identification (de Lecea *et al.* 1998, Sakurai *et al.* 1998)*
1999	Mutant mice generated (Chemelli *et al.* 1999)
1999	Association with narcolepsy revealed (Chemelli *et al.* 1999, Lin *et al.* 1999)*
2000	Lack of Hcrt neurones in human narcolepsy/cataplexy (Peyron *et al.* 2000, Thannickal *et al.* 2000)*
2001	Link with sympathetic activation (Shirasaka *et al.* 1999, Samson *et al.* 2002)
2003	Generation of Hcrt receptor knockout animals (Willie *et al.* 2003)
2000–2007	Identification of neurochemical activators/inhibitors (multiple authors)
2005	*In vivo* recordings of identified Hcrt neurones (Lee *et al.* 2005, Mileykovskiy *et al.* 2005)*
2005	Hcrt linked to brain reward function and addiction (Boutrel *et al.* 2005, Harris *et al.* 2005)*
2006	Anatomical afferents mapped (Sakurai *et al.* 2005, Yoshida *et al.* 2006)*
2007	Orally bioavailable Hcrt receptor antagonist (Brisbare-Roch *et al.* 2007)
2007	Optogenetic manipulation of Hcrt neurones (Adamantidis *et al.* 2007)

*Independent simultaneous discoveries.

Journal compilation © 2009 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2009.02004.x

a small number of direct projections from the suprachiasmatic nucleus (SCN) (Abrahamson *et al.* 2001), although most of the input is indirect through the DMH. In contrast, ascending projections mostly arrive from brainstem arousal centres. Evidence for these projections is thus far mainly provided by *in vitro* electrophysiological studies showing that acetylcholine produces depolarizing (excitatory) responses on Hcrt cells whereas serotonin and noradrenaline (NA) hyperpolarize Hcrt cell membranes (provide inhibition) (Li *et al.* 2002, Yamanaka *et al.* 2003b). Histamine neurones in the tuberomammillary nucleus are also targets of Hcrt neurones, and the reciprocal interactions between the histamine system and Hcrt may have an important role in cortical arousal (Haas *et al.* 2008). It is unclear, however, which population of Hcrt neurones is hyperpolarized by NA, as other reports suggest an excitatory influence for noradrenergic LC projections on Hcrt neurones (Bayer *et al.* 2005). In fact, differences in animal species might be responsible for these discrepancies. Several authors report a hyperpolarizing effect for NA on Hcrt cells in mice, whereas in rats NA depolarizes Hcrt neurones (Bayer *et al.* 2005). An interesting alternative might be that NA does both. As Grivel *et al.* (2005) suggested, NA is generally excitatory during the active period of the animal, but a switch from excitation to inhibition may occur when the animal is sleep deprived. The authors explain that wake active Hcrt cells are turned off during sleep deprivation to increase sleep pressure and thus help the organism to enter into the sleep state. Such findings are based on *in vitro* slice preparation studies and await further confirmation *in vivo*.

Additional projections to the hypothalamic Hcrt system have been inferred by the presence of receptors present in Hcrt cells, and we thus know that Hcrt neurones receive excitatory inputs from projections employing a variety of neurotransmitters such as glutamate (Li *et al.* 2002, Yamanaka *et al.* 2003a), ghrelin (Yamanaka *et al.* 2003b), glucagon-like peptide 1 (Acuna-Goycolea & van den Pol 2004), corticotrophin-releasing factor (Winsky-Sommerer *et al.* 2004), ATP (Wollmann *et al.* 2005), cholecystokinin, neurotensin, vasopressin and oxytocin (Tsujino *et al.* 2005), whereas additional inhibitory inputs are provided through GABA ($GABA_{a,b}$) (Li *et al.* 2002, Yamanaka *et al.* 2003a, Xie *et al.* 2006), glucose (Yamanaka *et al.* 2003a, Burdakov *et al.* 2005, 2006), serotonin (Yamanaka *et al.* 2003a, Muraki *et al.* 2004), dopamine (Yamanaka *et al.* 2003b), NPY (Fu *et al.* 2004), leptin (Yamanaka *et al.* 2003a) and adenosine (Liu & Gao 2007), inhibiting Hcrt neurones. In general, molecules that signal high energy inhibit Hcrt neurones, whereas low glucose, fatty acid and leptin concentrations activate Hcrt activity.

Arousal and hyperarousal

A direct consequence of the finding that Hcrt neurones receive inputs from specific neurotransmitter groups, such as corticotrophin-releasing factor, was the hypothesis that Hcrt neurones may be a direct target and an essential component of the acute stress response (Pañeda *et al.* 2005). This idea was consistent with behavioural hyperarousal that is associated with the fight-or-flight response, and the initial reports of central activation of the hypothalamo-pituitary-adrenal axis by Hcrt-1 infusion (Kuru *et al.* 2000, Jaszberenyi *et al.* 2001, Samson *et al.* 2002). In addition to CRF, input from limbic structures such as the amygdala and the bed nucleus of the stria terminalis may give Hcrt neurones a relevant role in the sympathetic activation during emotional stimuli. The link between Hcrt neurones and CRF also led us to hypothesize that Hcrt activity might be also important in behavioural states in which hyperarousal has a strong component, such as drug addiction. The first attempts to demonstrate this link were unsuccessful, as Hcrt-1 infusions did not change the amount or pattern of cocaine self-administration in rats. However, when the behaviour of addicted rats was extinguished, Hcrt-1 infusions were able to induce reinstatement of cocaine seeking (Boutrel *et al.* 2005). Moreover, a Hcrt receptor antagonist could block stress-induced reinstatement of cocaine and food-seeking behaviour, paving the way towards a possible new treatment to prevent relapse. These results have been confirmed by other groups (Harris *et al.* 2005), and the mechanisms by which Hcrt neurones influence drug-seeking behaviour are beginning to come to light (Borgland *et al.* 2006, Narita *et al.* 2006).

Animal models: loss and gain of function

In the late 1990s, loss-of-function studies in dogs (Lin *et al.* 1999) and mice (Chemelli *et al.* 1999) demonstrated that intact Hcrt signalling is essential for arousal stability, as lack of Hcrt ligand or Hcrtr 2 receptor in dogs causes narcolepsy with cataplexy. The studies with mutant animals that followed have confirmed the notion that Hcrtr2 receptor is necessary for balanced sleep architecture (Willie *et al.* 2003). A year later, Peyron *et al.* (2000) and Thannickal *et al.* (2000) independently demonstrated that narcoleptic patients have deficits in Hcrt mRNA expression and peptide content. These studies are indeed landmark discoveries that associated for the first time a restricted and non-redundant peptidergic system with disease, and have radically changed the perspective of sleep research. Recent excellent reviews have specifically addressed the role of Hcrt in narcolepsy and other sleep disorders (Scammell 2003). A direct consequence

 doi: 10.1111/j.1748-1716.2009.02004.x

of the association between lack of hcrt in narcolepsy is the development of diagnostic methods that are based on radioimmunoassays to Hcrt in cerebrospinal fluid and plasma. Also, Hcrt analogues have become an obvious target to treat the disease, and new small molecule Hcrt receptor antagonists are being used as therapeutic tools for insomnia and to prevent relapse of drug seeking (Brisbare-Roch *et al.* 2007).

How do Hcrt neurones maintain wakefulness? In 2005, the groups of Siegel and Jones independently reported the recording of identified Hcrt neurones from awake animals (Lee *et al.* 2005, Mileykovskiy *et al.* 2005). These recordings revealed that Hcrt neurones are phasically active during active waking, and practically silent during quiet waking and sleep. Interestingly, the highest activity was observed during transitions between rapid eye movement sleep and wakefulness. To further explore the mechanism by which phasic Hcrt activity maintains wakefulness, we used a newly developed optogenetic approach to manipulate the activity of Hcrt neurones *in vivo* (Adamantidis *et al.* 2007). These experiments demonstrated that photostimulation of Hcrt neurones expressing a light-sensitive cation channel, Channelrhodopsin 2, is sufficient to induce wakefulness, and therefore the main function of Hcrt neurones is to increase the firing probability of other arousal networks. Interestingly, chronic stimulation studies revealed that persistent stimulation of Hcrt neurones cannot maintain wakefulness (Adamantidis *et al.* 2007). It is thus likely that other neurotransmitter systems (e.g. NA, histamine or acetylcholine) will be responsible for sustained arousal.

Brain arousal (re)defined

Identifying the neurobiological substrates of arousal remains a key topic in neuroscience. Classical studies on the ascending reticular activating system identified a set of structures in the brainstem whose activity was essential for cortical desynchronization (Moruzzi & Magoun 1949). Lesion and pharmacology studies have indeed shed light on a set of nuclei that promote sleep and wakefulness. For instance, it is now widely accepted that inhibitory neurones from the ventrolateral pre-optic area in the hypothalamus are active during sleep and may be crucial to initiating sleep bouts (Sherin *et al.* 1996), and more recently, a subset of cortical interneurones has been identified as sleep active (Gerashchenko *et al.* 2008). These studies, however, rarely succeed at defining mechanistic relationships between neuronal circuits because the toxins and antagonists used are often non-selective. Also, an essential function such as arousal is likely to be mediated and modulated by multiple structures. Blanco-Centurion *et al.* (2007) showed that a combined lesion in cholinergic, histaminergic and adrenergic nuclei, but not lesions in individual nuclei, had stabilizing effects on sleep architecture. Therefore, the use of optogenetic tools is likely to increase our understanding of the contribution of each of the structures in the onset and maintenance of the states of vigilance (Deisseroth *et al.* 2006). Indeed, recent optogenetic manipulation of Hcrt neurones has spearheaded an effort to define interactions between genetically identified cell groups within the hypothalamus. As neurones containing MCH have now been recorded, and shown a pattern of unit activity that is complementary to Hcrt neurones (Hassani *et al.* 2009), future studies will involve optogenetic probing of Hcrt and MCH neurones to define their interactions. In addition to neurones, glia activity has recently been shown to affect sleep homeostasis (Halassa *et al.* 2009). The hypocretin system has also taught us that arousal circuits interact tightly with brain reward circuits, and with a dense network of metabolic sensor/effector neurones. These advances are likely to redefine the idea of arousal substrates and their role in behaviour.

Conflict of interest

There is no conflict of interest.

References

Abrahamson, E.E., Leak, R.K. & Moore, R.Y. 2001. The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. *Neuroreport* **12**, 435–440.

Acuna-Goycolea, C. & van den Pol, A. 2004. Glucagon-like peptide 1 excites hypocretin/orexin neurons by direct and indirect mechanisms: implications for viscera-mediated arousal. *J Neurosci* **24**, 8141–8152.

Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K. & de Lecea, L. 2007. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* **450**, 420–424.

Bayer, L., Eggermann, E., Serafin, M., Grivel, J., Machard, D., Muhlethaler, M. & Jones, B.E. 2005. Opposite effects of noradrenaline and acetylcholine upon hypocretin/orexin vs. melanin concentrating hormone neurons in rat hypothalamic slices. *Neuroscience* **130**, 807–811.

Blanco-Centurion, C., Gerashchenko, D. & Shiromani, P.J. 2007. Effects of saporin-induced lesions of three arousal populations on daily levels of sleep and wake. *J Neurosci* **27**, 14041–14048.

Borgland, S.L., Taha, S.A., Sarti, F., Fields, H.L. & Bonci, A. 2006. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* **49**, 589–601.

Boutrel, B., Kenny, P.J., Specio, S.E., Martin-Fardon, R., Markou, A., Koob, G.F. & de Lecea, L. 2005. Role for hypocretin in mediating stress-induced reinstatement of

Journal compilation © 2009 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2009.02004.x

cocaine-seeking behavior. *Proc Natl Acad Sci USA* **102**, 19168–19173.

Brisbare-Roch, C., Dingemanse, J., Koberstein, R., Hoever, P., Aissaoui, H., Flores, S., Mueller, C., Nayler, O., van Gerven, J., de Haas, S.L. *et al.* 2007. Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nat Med* **13**, 150–155.

Burdakov, D., Gerasimenko, O. & Verkhratsky, A. 2005. Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *J Neurosci* **25**, 2429–2433.

Burdakov, D., Jensen, L.T., Alexopoulos, H., Williams, R.H., Fearon, I.M., O'Kelly, I., Gerasimenko, O., Fugger, L. & Verkhratsky, A. 2006. Tandem-pore K^+ channels mediate inhibition of orexin neurons by glucose. *Neuron* **50**, 711–722.

Chemelli, R.M., Willie, J.T., Sinton, C.M., Elmquist, J.K., Scammell, T., Lee, C., Richardson, J.A., Williams, S.C., Xiong, Y., Kisanuki, Y., Fitch, T.E., Nakazato, M., Hammer, R.E., Saper, C.B. & Yanagisawa, M. 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98**, 437–451.

Chen, C.T., Dun, S.L., Kwok, E.H., Dun, N.J. & Chang, J.K. 1999. Orexin A-like immunoreactivity in the rat brain. *Neurosci Lett* **260**, 161–164.

Date, Y., Ueta, Y., Yamashita, H., Yamaguchi, H., Matsukura, S., Kangawa, K., Sakurai, T., Yanagisawa, M. & Nakazato, M. 1999. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. *Proc Natl Acad Sci USA* **96**, 748–753.

Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A. & Schnitzer, M.J. 2006. Next-generation optical technologies for illuminating genetically targeted brain circuits. *J Neurosci* **26**, 10380–10386.

Fu, L.-Y., Acuna-Goycolea, C. & van den Pol, A.N. 2004. Neuropeptide Y inhibits hypocretin/orexin neurons by multiple pre-synaptic and post-synaptic mechanisms: tonic depression of the hypothalamic arousal system. *J Neurosci* **24**, 8741–8751.

Gautvik, K.M., de Lecea, L., Gautvik, V.T., Danielson, P.E., Tranque, P., Dopazo, A., Bloom, F.E. & Sutcliffe, J.G. 1996. Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. *Proc Natl Acad Sci USA* **93**, 8733–8738.

Gerashchenko, D., Wisor, J.P., Burns, D., Reh, R.K., Shiromani, P.J., Sakurai, T., de la Iglesia, H.O. & Kilduff, T.S. 2008. Identification of a population of sleep-active cerebral cortex neurons. *Proc Natl Acad Sci USA* **105**, 10227–10232.

Grivel, J., Cvetkovic, V., Bayer, L., Machard, D., Tobler, I., Muhlethaler, M. & Serafin, M. 2005. The wake-promoting hypocretin/orexin neurons change their response to noradrenaline after sleep deprivation. *J Neurosci* **25**, 4127–4130.

Haas, H.L., Sergeeva, O.A. & Selbach, O. 2008. Histamine in the nervous system. *Physiol Rev* **88**, 1183–1241.

Halassa, M.M., Florian, C., Fellin, T., Munoz, J.R., Lee, S.Y., Abel, T., Haydon, P.G. & Frank, M.G. 2009. Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* **61**, 213–219.

Harris, G.C., Wimmer, M. & Jones, G.A. 2005. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* **437**, 556–559.

Hassani, O.K., Lee, M.G. & Jones, B.E. 2009. Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle. *Proc Natl Acad Sci USA* **106**, 2418–2422.

Jaszberenyi, M., Bujdoso, E. & Telegdy, G. 2001. The role of neuropeptide y in orexin-induced hypothalamic-pituitary-adrenal activation. *J Neuroendocrinol* **13**, 438–441.

Kuru, M., Ueta, Y., Serino, R., Nakazato, M., Yamamoto, Y., Shibuya, I. & Yamashita, H. 2000. Centrally administered orexin/hypocretin activates HPA axis in rats. *Neuroreport* **11**, 1977–1980.

de Lecea, L. & Sutcliffe, J.G. (eds) 2005. *Hypocretins: Integrators of Physiological Functions*. Springer, New York.

de Lecea, L., Kilduff, T.S., Peyron, C., Gao, X., Foye, P.E., Danielson, P.E., Fukuhara, C., Battenberg, E.L., Gautvik, V.T., Bartlett, F.S., 2nd, Frankel, W.N., van den Pol, A.N., Bloom, F.E., Gautvik, K.M. & Sutcliffe, J.G. 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA* **95**, 322–327.

Lee, M.G., Hassani, O.K. & Jones, B.E. 2005. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J Neurosci* **25**, 6716–6720.

Li, Y., Gao, X.-B., Sakurai, T. & van den Pol, A.N. 2002. Hypocretin/orexin excites hypocretin neurons via a local glutamate neuron: a potential mechanism for orchestrating the hypothalamic arousal system. *Neuron* **36**, 1169–1181.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., Qiu, X., de Jong, P.J., Nishino, S. & Mignot, E. 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**, 365–376.

Liu, Z.-W. & Gao, X.-B. 2007. Adenosine inhibits activity of hypocretin/orexin neurons by the a1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. *J Neurophysiol* **97**, 837–848.

Mileykovskiy, B.Y., Kiyashchenko, L.I. & Siegel, J.M. 2005. Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron* **46**, 787–798.

Moruzzi, G. & Magoun, H.W. 1949. Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol* **1**, 455–473.

Muraki, Y., Yamanaka, A., Tsujino, N., Kilduff, T.S., Goto, K. & Sakurai, T. 2004. Serotonergic regulation of the orexin/hypocretin neurons through the 5-HT1A receptor. *J Neurosci* **24**, 7159–7166.

Nambu, T., Sakurai, T., Mizukami, K., Hosoya, Y., Yanagisawa, M. & Goto, K. 1999. Distribution of orexin neurons in the adult rat brain. *Brain Res* **827**, 243–260.

Narita, M., Nagumo, Y., Hashimoto, S., Khotib, J., Miyatake, M., Sakurai, T., Yanagisawa, M., Nakamachi, T., Shioda, S. & Suzuki, T. 2006. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J Neurosci* **26**, 398–405.

Pañeda, C., Winsky-Sommerer, R., Boutrel, B. & de Lecea, L. 2005. The corticotropin-releasing factor-hypocretin connection: implications in stress response and addiction. *Drug News Perspect* **18**, 250–255.

Peyron, C., Tighe, D.K., van den Pol, A.N., de Lecea, L., Heller, H.C., Sutcliffe, J.G. & Kilduff, T.S. 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* **18**, 9996–10015.

Peyron, C., Faraco, J., Rogers, W., Ripley, B., Overeem, S., Charnay, Y., Nevsimalova, S., Aldrich, M., Reynolds, D., Albin, R. *et al.* 2000. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6**, 991–997.

Sakurai, T. 1999. Orexins and orexin receptors: implication in feeding behavior. *Regul Pept* **85**, 25–30.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richarson, J.A., Kozlowski, G.P., Wilson, S. *et al.* 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92**, 1 page following 696.

Sakurai, T., Nagata, R., Yamanaka, A., Kawamura, H., Tsujino, N., Muraki, Y., Kageyama, H., Kunita, S., Takahashi, S., Goto, K., Koyama, Y., Shioda, S. & Yanagisawa, M. 2005. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* **46**, 297–308.

Samson, W.K., Taylor, M.M., Follwell, M. & Ferguson, A.V. 2002. Orexin actions in hypothalamic paraventricular nucleus: physiological consequences and cellular correlates. *Regul Pept* **104**, 97–103.

Scammell, T.E. 2003. The neurobiology, diagnosis, and treatment of narcolepsy. *Ann Neurol* **53**, 154–166.

Sherin, J.E., Shiromani, P.J., McCarley, R.W. & Saper, C.B. 1996. Activation of ventrolateral preoptic neurons during sleep. *Science* **271**, 216–219.

Shirasaka, T., Nakazato, M., Matsukura, S., Takasaki, M. & Kannan, H. 1999. Sympathetic and cardiovascular actions of orexins in conscious rats. *Am J Physiol* **277**, R1780–R1785.

Thannickal, T.C., Moore, R.Y., Nienhuis, R., Ramanathan, L., Gulyani, S., Aldrich, M., Cornford, M. & Siegel, J.M. 2000. Reduced number of hypocretin neurons in human narcolepsy. *Neuron* **27**, 469–474.

Tsujino, N., Yamanaka, A., Ichiki, K., Muraki, Y., Kilduff, T.S., Yagami, K.-i., Takahashi, S., Goto, K. & Sakurai, T. 2005. Cholecystokinin activates orexin/hypocretin neurons through the cholecystokinin A receptor. *J Neurosci* **25**, 7459–7469.

Usui, H., Falk, J.D., Dopazo, A., de Lecea, L., Erlander, M.G. & Sutcliffe, J.G. 1994. Isolation of clones of rat striatum-specific mRNAs by directional tag PCR subtraction. *J Neurosci* **14**, 4915–4926.

Willie, J.T., Chemelli, R.M., Sinton, C.M., Tokita, S., Williams, S.C., Kisanuki, Y.Y., Marcus, J.N., Lee, C., Elmquist, J.K., Kohlmeier, K.A., Leonard, C.S., Richardson, J.A., Hammer, R.E. & Yanagisawa, M. 2003. Distinct narcolepsy syndromes in orexin receptor-2 and orexin null mice: molecular genetic dissection of non-REM and REM sleep regulatory processes. *Neuron* **38**, 715–730.

Winsky-Sommerer, R., Yamanaka, A., Diano, S., Borok, E., Roberts, A.J., Sakurai, T., Kilduff, T.S., Horvath, T.L. & de Lecea, L. 2004. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *J Neurosci* **24**, 11439–11448.

Wollmann, G., Acuna-Goycolea, C. & van den Pol, A.N. 2005. Direct excitation of hypocretin/orexin cells by extracellular ATP at P2X receptors. *J Neurophysiol* **94**, 2195–2206.

Xie, X., Crowder, T.L., Yamanaka, A., Morairty, S.R., LeWinter, R.D., Sakurai, T. & Kilduff, T.S. 2006. GABAB receptor-mediated modulation of hypocretin/orexin neurones in mouse hypothalamus. *J Physiol* **574**, 399–414.

Yamanaka, A., Beuckmann, C.T., Willie, J.T., Hara, J., Tsujino, N., Mieda, M., Tominaga, M., Yagami, K.-i., Sugiyama, F., Goto, K., Yanagisawa, M. & Sakurai, T. 2003a. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* **38**, 701–713.

Yamanaka, A., Muraki, Y., Tsujino, N., Goto, K. & Sakurai, T. 2003b. Regulation of orexin neurons by the monoaminergic and cholinergic systems. *Biochem Biophys Res Commun* **303**, 120–129.

Yoshida, K., McCormack, S., Espana, R.A., Crocker, A. & Scammell, T.E. 2006. Afferents to the orexin neurons of the rat brain. *J Comp Neurol* **494**, 845–861.

Journal compilation © 2009 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2009.02004.x