PubMed Central
HHMI
HOWARD HUGHES MEDICAL INSTITUTE
AUTHOR MANUSCRIPT
Accepted for publication in a peer-reviewed journal

Published as: *Acta Physiol (Oxf)*. 2010 March ; 198(3): 287–294.

Histamine-1 receptor is not required as a downstream effector of orexin-2 receptor in maintenance of basal sleep/wake states

M. Hondo[1,2], **K. Nagai**[2], **K. Ohno**[2], **Y. Kisanuki**[3], **J. T. Willie**[3], **T. Watanabe**[4], **M. Yanagisawa**[3], and **T. Sakurai**[1,2]

[1]Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan

[2]Department of Pharmacology, Institute of Basic Medical Science, University of Tsukuba, Tsukuba, Ibaraki, Japan

[3]Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

[4]Center for Innovation in Immunoregulative Technology and Therapeutics, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto, Japan

Abstract

Aim—The effect of orexin on wakefulness has been suggested to be largely mediated by activation of histaminergic neurones in the tuberomammillary nucleus (TMN) via orexin receptor-2 (OX_2R). However, orexin receptors in other regions of the brain might also play important roles in maintenance of wakefulness. To dissect the role of the histaminergic system as a downstream mediator of the orexin system in the regulation of sleep/wake states without compensation by the orexin receptor-1 (OX_1R) mediated pathways, we analysed the phenotype of *Histamine-1 receptor (H_1R)* and OX_1R double-deficient ($H_1R^{-/-};OX_1R^{-/-}$) mice. These mice lack OX_1R-mediated pathways in addition to deficiency of H_1R, which is thought to be the most important system in downstream of OX_2R.

Methods—We used H_1R deficient ($H_1R^{-/-}$) mice, $H_1R^{-/-};OX_1R^{-/-}$ mice, OX_1R and OX_2R double-deficient ($OX_1R^{-/-};OX_2R^{-/-}$) mice, and wild type controls. Rapid eye movement (REM) sleep, non-REM (NREM) sleep and awake states were determined by polygraphic electroencephalographic/electromyographic recording.

Results—No abnormality in sleep/wake states was observed in $H_1R^{-/-}$ mice, consistent with previous studies. $H_1R^{-/-};OX_1R^{-/-}$ mice also showed a sleep/wake phenotype comparable to that of wild type mice, while $OX_1R^{-/-}$; $OX_2R^{-/-}$ mice showed severe fragmentation of sleep/wake states.

Conclusion—Our observations showed that regulation of the sleep/wake states is completely achieved by OX_2R-expressing neurones without involving H_1R-mediated pathways. The maintenance of basal physiological sleep/wake states is fully achieved without both H_1 and OX_1 receptors. Downstream pathways of OX_2R other than the histaminergic system might play an important role in the maintenance of sleep/wake states.

*T. Sakurai, Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine, Kanazawa University, Kanazawa, Ishikawa 920-8640, Japan. tsakurai@med.kanazawa-u.ac.jp.

HHMI Author Manuscript
HHMI Author Manuscript
HHMI Author Manuscript

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

Keywords

electroencephalography; histamine H_1 receptor; orexin receptor-1; orexin receptor-2; sleep/wake states; tuberomammillary nucleus

---

Recent studies on the efferent and afferent systems of orexin/hypocretin neurones, and phenotypic characterization of mice with genetic modification of the orexin system have suggested roles of the orexin system in regulation of sleep and wakefulness through interactions with systems that regulate emotion, the reward system and energy homeostasis (Yamanaka *et al.* 2003, Akiyama *et al.* 2004, Mieda *et al.* 2004, Boutrel *et al.* 2005, Harris *et al.* 2005, Sakurai *et al.* 2005, Narita *et al.* 2006, Yoshida *et al.* 2006). There are two orexin receptor subtypes, named orexin receptor-1 (OX_1R) and orexin receptor-2 (OX_2R) (Sakurai *et al.* 1998). Orexin-producing neurones, localized in the lateral hypothalamic area (LHA), send projections to all over the central nervous system except the cerebellum. Especially dense orexin-immunoreactive fibres are found in monoaminergic and cholinergic nuclei in the brain stem regions (Marcus *et al.* 2001). Moreover, these nuclei abundantly express orexin receptors.

Mice with targeted deletion of the *prepro-orexin* gene (*Orexin*$^{-/-}$ mice) display a phenotype strikingly similar to human narcolepsy (Chemelli *et al.* 1999). Besides, functionally null mutations in the OX_2R gene were found in familial narcoleptic dogs (Lin *et al.* 1999). Consistently, $OX_2R^{-/-}$ mice show fragmented sleep/wake behaviour and direct transitions from wakefulness to rapid eye movement (REM) sleep, although the phenotype is significantly milder than that of *Orexin*$^{-/-}$ mice, whereas $OX_1R^{-/-}$ mice do not have any overt behavioural abnormalities (Willie *et al.* 2001). These observations suggest that the OX_2R-mediated pathway has pivotal roles, while OX_1R has an additional role in the regulation of sleep/wake states. Narcoleptic human brains have been shown to contain markedly reduced numbers of orexin neurones (Peyron *et al.* 2000, Thannickal *et al.* 2000).

Orexin receptors are distributed in a pattern consistent with orexin projections. mRNAs for OX_1R and OX_2R are differentially expressed throughout the brain (Marcus *et al.* 2001). OX_1R mRNA is most abundantly expressed in the locus coeruleus (LC), whereas OX_2R mRNA is most abundantly expressed in the histaminergic tuberomammillary nucleus (TMN). The raphe nuclei and laterodorsal/pedunculopontine tegmental nuclei (LDT/PPT) contain mRNA for both receptors. *In vitro* electrophysiological studies have also shown that all these monoaminergic/cholinergic neurones are activated by orexins (Sakurai 2007). These monoaminergic/cholinergic neurones are implicated in regulation of sleep/wake states (Vanni-Mercier *et al.* 1984).

Huang *et al.* (2001) suggested that the arousal effect of orexin A largely depends on activation of histaminergic neurotransmission mediated by histamine receptor-1 (H_1R). This observation suggests the importance of H_1R as the downstream player of orexin system. Consistently, the histamine concentration in the brain is decreased in OX_2R-mutated narcoleptic dogs (Lin *et al.* 1999).

Histaminergic neurones are exclusively localized in the TMN (Watanabe *et al.* 1984), and project to practically all brain regions, with especially dense innervations in the hypothalamus, basal forebrain and amygdala (Panula & Costa 1984, Takeda *et al.* 1984). It has been reported that the firing rates of histamine neurones vary across the sleep/wake cycle (Sakai *et al.* 1990), and intracerebroventricular (i.c.v.) injection of histamine or a H_1R agonist induces wakefulness and diminution of non-REM (NREM) sleep (Monti *et al.* 1986,

HHMI Author Manuscript

HHMI Author Manuscript

HHMI Author Manuscript

Tasaka *et al.* 1989, Monti 1993). These results suggest that the brain histaminergic system plays a critical role in regulation of sleep/wake states through activation of H_1R.

Orexin neurones densely innervate TMN neurones, in which OX_2R is abundantly expressed (Marcus *et al.* 2001), further suggesting the possibility that orexin neurones regulate vigilance and arousal through modulating the activity of histaminergic neurones in the TMN.

In the present study, we analysed the role of the H_1R as downstream signalling of the OX_2R-mediated pathway, using H_1R and OX_1R double-deficient mice ($H_1R^{-/-};OX_1R^{-/-}$). In these mice, the OX_1R-mediated pathway was totally abolished, while the OX_2R-mediated pathway remained intact, but the H_1R-mediated pathway was deficient. Therefore, these mice enable us to analyse the roles of the H_1R-mediated pathway as the downstream mediator of orexins without compensation by OX_1R-mediated pathways.

Materials and methods

Animals

All experimental procedures involving animals were conducted with the approval of the University of Tsukuba and Kanazawa University Animal Care Committees. All efforts were made to minimize animal suffering and to limit the number of animals used. Procedures related to handling of $H_1R^{-/-}$ mice and $OX_1^{-/-}$ and $OX_2R^{-/-}$ mice have been described in detail previously (Inoue *et al.* 1996).$OX_1R^{/-}$ and $OX_2R^{/-}$ mice were also described previously (Willie *et al.* 2001, 2003). $H_1R^{+/-};OX_1R^{+/-}$ mice were obtained by mating $H_1R^{-/-}$ mice and $OX_1R^{-/-}$ mice; then we crossed them to obtain $H_1R^{-/-};OX_1R^{-/-}$ mice. As a wild type control mice, we used littermates of $H_1R^{-/-}$ mice. Mice were housed under controlled lighting (12 h light–dark cycle; light on at 08:00 hours, off at 20:00 hours) and temperature conditions. Food and water were available *ad libitum*. All mice were backcrossed with C57BL/6J mice at least six times. We analysed $H_1R^{-/-}$ ($n = 4$), $H_1R^{-/-};OX_1R^{-/-}$ ($n = 4$), $OX_1R^{-/-};OX_2R^{-/-}$ ($n = 5$) and wild type mice ($n = 5$).

Surgery

Male mice (10–12 weeks old, 20–25 g at the time of surgery) were prepared for chronic monitoring of electroencephalographic/electromyographic (EEG/EMG) signals using a lightweight implant and cabling procedure. Full details of this technique have been published previously (Chemelli *et al.* 1999). Briefly, the EEG/EMG implant was based on a six-pin double inline microcomputer connector, modified to form four EEG electrodes, each 1.3 mm × 0.3 mm (h × w), positioned 4.6 mm × 2.9 mm (l × w) apart, with two EMG electrodes soldered to the entry pins. Mice were anaesthetized with sodium pentobarbital (Nembutal, 50–60 mg kg^{-1} i.p.), and standard sterile surgical and stereotaxic procedures were employed for implant placement. Four burr holes were drilled in the cranium, anterior and posterior to the bregma, bilaterally (AP 1.1, ML 1.4 and AP-3.6, ML1.4) according to the atlas of Franklin & Paxinos (1997). The implant was then inserted into these holes and fixed to the skull with adhesive dental cement, the EMG electrodes were placed into the nuchal musculature and the wounds were closed with sutures.

Sleep recording

Immediately after surgery, mice were housed singly for a recovery period of 1 week. The head-mounted connector was coupled via a lightweight cable to a slip ring commutator, which was suspended from a counterbalanced arm mounted on a standard shoebox cage (19 cm × 30 cm; Allentown Caging, Allentown, NJ, USA). This allowed mice full freedom of movement. The cage was modified to provide side delivery of food and water, which were

HHMI Author Manuscript

available *ad libitum*. All mice were habituated to these conditions for at least 7 days before the start of recording. Then, EEG/EMG recording for two consecutive 24 h periods, beginning at lights on at 20:00 hours was performed. Infrared video recording was simultaneously performed. EEG/EMG signals were amplified using a multichannel amplifier (Nihon Koden, Tokyo, Japan) and filtered (EEG: 0.5–100 Hz; EMG: 0.5–100 Hz) before being digitized at a sampling rate of 250 Hz, displayed on a paperless polygraph system and archived for off-line sleep staging and analysis.

Sleep scoring and data analysis

EEG/EMG records were visually scored into 16-s epochs of awake, REM sleep and NREM sleep. Data were analysed by two-way ANOVA followed by *post-hoc* analysis of significance with Bonferroni's or Student's *t*-test using the Stat View 5.0 software package (Abacus Concepts, Berkeley, CA, USA). In all cases, $P < 0.001$ was taken as the level of significance.

Results

Sleep state patterns of wild type mice, $H_1R^{-/-}$ mice, $H_1R^{-/-};OX_1R^{-/-}$ mice and $OX_1R^{-/-};OX_2R^{-/-}$ mice were revealed by simultaneous EEG/EMG recording as reported previously (Chemelli *et al.* 1999). Typical representative 12 h dark period (20:00 hours to 08:00 hours) hypnograms for wild type mice, $H_1R^{-/-}$ mice, $H_1R^{-/-};OX_1R^{-/-}$ mice and $OX_1R^{-/-};OX_2R^{-/-}$ mice are shown in Figure 1. Under baseline conditions, the patterns of sleep/wake states were not statistically different among wild type mice, $H_1R^{-/-}$ mice and $H_1R^{-/-}; OX_1R^{-/-}$ mice (Fig. 2, Table 1). There were no significant differences between wild type mice, $H_1R^{-/-}$ mice and $H_1R^{-/-};OX_1R^{-/-}$ mice, in both the amount and duration of NREM sleep, REM sleep and wakefulness during the dark period (Fig. 2). These parameters were not distinguishable among any of the genotypes during the light period. Hourly analysis of quantities of NREM sleep, REM sleep and wakefulness also revealed no differences between wild type mice, $H_1R^{-/-}$ mice and $H_1R^{-/-};OX_1R^{-/-}$ mice (Fig. 3). In contrast, $OX_1R^{-/-};OX_2R^{-/-}$ mice showed severe fragmentation of sleep/wakefulness states, reflected in significantly shorter duration of wakefulness, and frequent direct transitions from wakefulness to REM sleep, as described previously (Willie *et al.* 2003) (Figs 1 and 2). $OX_1R^{-/-};OX_2R^{-/-}$ mice showed abnormal circadian distribution of REM sleep (Fig. 3). These phenotypes are similar to those of *Orexin*$^{-/-}$ mice (Chemelli *et al.* 1999).

Discussion

The actions of orexins are mediated via two G protein-coupled receptors, OX_1R and OX_2R. Although these orexin receptors are expressed in a pattern consistent with orexin projections, mRNAs for OX_1R and OX_2R are differentially distributed in the brain, suggesting their distinct roles. Several studies have indicated that the effect of orexin is largely or partially mediated by activation of the histaminergic H_1R in the downstream of OX_2R in the TMN (Eriksson *et al.* 2001, Huang *et al.* 2001, Yamanaka *et al.* 2002, Shigemoto *et al.* 2004). Consistently, $OX_2R^{-/-}$ mice are affected by cataplexy-like attacks of REM sleep and sleep/wake fragmentation (Willie *et al.* 2001, Sakurai 2007), although the phenotype of $OX_2R^{-/-}$ mice was significantly less severe than that of $OX_1R^{-/-};OX_2R^{-/-}$ mice (Willie *et al.* 2003, Sakurai 2007). From these observations, the histaminergic pathway is currently thought to be the important link in the OX_2R pathway in arousal regulation.

Three distinct subtypes of histamine receptors, H_1 receptor (H_1R), H_2 receptor (H_2R) and H_3 receptor (H_3R), are distributed in the brain and exhibit well-defined distribution patterns (Bouthenet *et al.* 1988, Martinez-Mir *et al.* 1990). H_1R and H_2R are post-synaptic receptors coupled to $G_{q/11}$ and G_s protein, and H_3R is a pre-synaptic autoreceptor coupled to $G_{i/o}$

HHMI Author Manuscript

HHMI Author Manuscript

HHMI Author Manuscript

protein. Huang *et al.* 2001 showed that the arousal effect of orexin A almost totally depends on activation of histaminergic neurotransmission mediated by H_1R. However, Huang *et al.* 2006 also reported that $H_1R^{-/-}$ mice showed sleep/wake states essentially identical to those of wild type mice but with fewer incidents of brief awakening (<16 s epochs), and prolonged durations of NREM sleep episodes, although the physiological significance of brief awakening is not yet well understood (Huang *et al.* 2006). This suggests that the physiological function of H_1R does not contribute to regulation of the basal amount of sleep/wake states. Besides, histidine decarboxylase (HDC)-deficient mice showed clear sleep/wake phenotypes, suggesting the possible involvement of H_2R and H_3R in physiological regulation of sleep/wake states (Parmentier *et al.* 2002). However, previous pharmacological studies demonstrated that the clinical use of H_1R antagonists induces sleepiness (Nicholson *et al.* 1985). Also, slow-wave sleep is induced in cats by microinjection of the selective H_1R antagonist pyrilamine into the pre-optic area (Lin & Jouvet 1994) or the dorsal pontine tegmentum (Lin *et al.* 1996). These results suggest that H_1R mediates the waking effect of histamine. These observations suggest a possibility that the normal sleep/wake phenotype of $H_1R^{-/-}$ mice is compensated by other systems such as OX_1R-mediated activation of the noradrenergic neurones in the LC.

In this study, we analysed $H_1R^{-/-};OX_1R^{-/-}$ mice to define the importance of the role of H_1R as a downstream effector of the orexinergic system in sleep/wake regulation. These mice lack both OX_1R-mediated regulation of noradrenergic neurones in the LC, and the H_1R system belonging to downstream of OX_2R in the TMN. Signalling pathways in downstream of OX_2R, such as serotonergic neurones in the raphe nuclei (Brown *et al.* 2002), cholinergic neurones in the LDT/PPT (Burlet *et al.* 2002), and dopaminergic neurones in the ventral tegmental area (VTA) remain intact (Korotkova *et al.* 2003). The raphe nuclei, LDT/PPT and VTA also abundantly express OX_1R in wild type mice, but in $H_1R^{-/-};OX_1R^{-/-}$ mice, only OX_2R is expressed in these regions. By using these mice, we analysed the role of the H_1R system belonging to downstream of OX_2R. We found that $H_1R^{-/-};OX_1R^{-/-}$ mice showed a normal sleep/wake phenotype compared with wild type mice. These results are not consistent with the hypothesis that 'activation of H_1R in the downstream of OX_2R is the most important pathway in sleep/wake regulation by orexin'. Consistent with our present results, Blanco-Centurion *et al.* (2007) reported that saporin-induced lesions in histaminergic neurones in the TMN, along with cholinergic neurones in the basal forebrain (BF), and noradrenergic neurones in the LC did not change the waking amount, although the lesions affected the transition time of the light-dark phase (Blanco-Centurion 2007). Our observations suggest that regulation of basal physiological sleep/wake states is fully achieved without H_1R even when OX_1R is absent. Loss of the H_1R-mediated pathway might be fully compensated by OX_2R-mediated pathways without a contribution of OX_1R. Considering the fact that $OX_2R^{-/-}$ show a mild but definite narcoleptic phenotype, OX_2R-mediated pathways other than histaminergic pathways might be highly important for basal regulation of sleep/wake states.

Acknowledgments

The authors thank Dr Wendy Gray for critical reading the manuscript. This study was supported in part by a grant-in-aid for scientific research (S, A, B) and the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan, Mitsui Life Social Welfare Foundation, Takeda Science Foundation.

References

Akiyama M, Yuasa T, Hayasaka N, Horikawa K, Sakurai T, Shibata S. Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. Eur J Neurosci. 2004; 20:3054–3062. [PubMed: 15579160]

HHMI Author Manuscript

HHMI Author Manuscript

HHMI Author Manuscript

Blanco-Centurion C, Gerashchenko D, Shiromani PJ. Effects of saporin-induced lesions of three arousal populations on daily levels of sleep and wake. J Neurosci. 2007; 27:14041–14048. [PubMed: 18094243]

Bouthenet ML, Ruat M, Sales N, Garbarg M, Schwartz JC. A detailed mapping of histamine H1-receptors in guinea-pig central nervous system established by autoradiography with [125I]iodobolpyramine. Neuroscience. 1988; 26:553–600. [PubMed: 3173689]

Boutrel B, Kenny PJ, Specio SE, Martin-Fardon R, Markou A, Koob GF, De Lecea L. Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. Proc Natl Acad Sci USA. 2005; 102:19168–19173. [PubMed: 16357203]

Brown RE, Sergeeva OA, Eriksson KS, Haas HL. Convergent excitation of dorsal raphe serotonin neurons by multiple arousal systems (orexin/hypocretin, histamine and noradrenaline). J Neurosci. 2002; 22:8850–8859. [PubMed: 12388591]

Burlet S, Tyler CJ, Leonard CS. Direct and indirect excitation of laterodorsal tegmental neurons by hypocretin/orexin peptides: implications for wakefulness and narcolepsy. J Neurosci. 2002; 22:2862–2872. [PubMed: 11923451]

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell TE, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 1999; 98:437–451. [PubMed: 10481909]

Eriksson KS, Sergeeva O, Brown RE, Haas HL. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci. 2001; 21:9273–9279. [PubMed: 11717361]

Franklin, KBJ.; Paxinos, G. The mouse brain in Stereotaxic Coordinates. 2. Academic Press; San Diego: 1997.

Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. Nature. 2005; 437:556–559. [PubMed: 16100511]

Huang ZL, Mochizuki T, Qu WM, Hong ZY, Watanabe T, Urade Y, Hayaishi O. Altered sleep-wake characteristics and lack of arousal response to H3 receptor antagonist in histamine H1 receptor knockout mice. Proc Natl Acad Sci USA. 2006; 103:4687–4692. [PubMed: 16537376]

Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N, Watanabe T, Urade Y, Hayaishi O. Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA. 2001; 98:9965–9970. [PubMed: 11493714]

Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T, Watanabe T. Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proc Natl Acad Sci USA. 1996; 93:13316–13320. [PubMed: 8917588]

Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. J Neurosci. 2003; 23:7–11. [PubMed: 12514194]

Lin JS, Sakai K, Jouvet M. Hypothalamo-preoptic histaminergic projections in sleep-wake control in the cat. Eur J Neurosci. 1994; 6:618–625. [PubMed: 8025714]

Lin JS, Hou Y, Sakai K, Jouvet M. Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. J Neurosci. 1996; 16:1523–1537. [PubMed: 8778302]

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, De Jong PJ, Nishino S, Mignot E. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell. 1999; 98:365–376. [PubMed: 10458611]

Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, Elmquist JK. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol. 2001; 435:6–25. [PubMed: 11370008]

Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM. Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates. Brain Res. 1990; 526:322–327. [PubMed: 1979518]

Mieda M, Williams SC, Sinton CM, Richardson JA, Sakurai T, Yanagisawa M. Orexin neurons function in an efferent pathway of a food-entrainable circadian oscillator in eliciting food-anticipatory activity and wakefulness. J Neurosci. 2004; 24:10493–10501. [PubMed: 15548664]

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

Monti J. Involvement of histamine in the control of the waking state. Life Sci. 1993; 53:1331–1338. [PubMed: 8412498]

Monti JM, Pellejero T, Jantos H. Effects of H1- and H2-histamine receptor agonists and antagonists on sleep and wakefulness in the rat. J Neural Transm. 1986; 66:1–11. [PubMed: 3734773]

Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, Sakurai T, Yanagisawa M, Nakamachi T, Shioda S, Suzuki T. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. J Neurosci. 2006; 26:398–405. [PubMed: 16407535]

Nicholson AN, Pascoe PA, Stone BM. Histaminergic systems and sleep. Studies in man with H1 and H2 antagonists. Neuropharmacology. 1985; 24:245–250. [PubMed: 2859543]

Panula PYH, Costa E. Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA. 1984; 81:2572–2576. [PubMed: 6371818]

Parmentier R, Ohtsu H, Djebbara-Hannas Z, Valatx JL, Watanabe T, Lin JS. Anatomical, physiological, and pharmacological characteristics of histidine decarboxylase knock-out mice: evidence for the role of brain histamine in behavioral and sleep-wake control. J Neurosci. 2002; 22:7695–7711. [PubMed: 12196593]

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Nevsimalova S, Aldrich M, Reynolds D, Albin R, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med. 2000; 9:991–997. [PubMed: 10973318]

Sakai, K.; El Mansari, M.; Lin, JS.; Zhang, JG.; Vanni Mercier, G. The posterior hypothalamus in the regulation of wakefulness and paradoxical sleep. In: Mancia, M.; Marini, G., editors. The Diencephalon and Sleep. Raven Press; New York: 1990. p. 171-198.

Sakurai T. The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. Nat Rev Neurosci. 2007; 8:171–181. [PubMed: 17299454]

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell. 1998; 92:573–585. [PubMed: 9491897]

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. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. Neuron. 2005; 46:297–308. [PubMed: 15848807]

Shigemoto Y, Fujii Y, Shinomiya K, Kamei C. Participation of histaminergic H1 and noradrenergic alpha 1 receptors in orexin A-induced wakefulness in rats. Brain Res. 2004; 1023:121–125. [PubMed: 15364026]

Takeda N, Inagaki S, Taguchi Y, Tohyama M, Watanabe T, Wada H. Origins of histamine-containing fibers in the cerebral cortex of rats studied by immunohistochemistry with histidine decarboxylase as a marker and transection. Brain Res. 1984; 323:55–63. [PubMed: 6098336]

Tasaka K, Chung YH, Sawada K. Excitatory effect of histamine on EEGs of the cortex and thalamus in rats. Agents Actions. 1989; 27:127–130. [PubMed: 2568736]

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. Reduced number of hypocretin neurons in human narcolepsy. Neuron. 2000; 27:469–474. [PubMed: 11055430]

Vanni-Mercier G, Sakai K, Jouvet M. Specific neurons for wakefulness in the posterior hypothalamus in the cat. C R Acad Sci III. 1984; 298:195–200. [PubMed: 6424901]

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, Tohyama M, Wada H. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res. 1984; 295:13–25. [PubMed: 6713171]

Willie JT, Chemelli RM, Sinton CM, Yanagisawa M. To eat or to sleep? Orexin in the regulation of feeding and wakefulness. Annu Rev Neurosci. 2001; 24:429–458. [PubMed: 11283317]

Willie JT, Chemelli RM, Sinton CM, Tokita S, Williams SC, Kisanuki YY, Marcus JN, Lee C, Elmquist JK, Kohlmeier KA, Leonard CS, Richardson JA, Hammer RE, Yanagisawa M. Distinct

HHMI Author Manuscript

HHMI Author Manuscript

HHMI Author Manuscript

narcolepsy syndromes in Orexin receptor-2 and Orexin null mice: molecular genetic dissection of Non-REM and REM sleep regulatory processes. Neuron. 2003; 38:715–730. [PubMed: 12797957]

Yamanaka A, Tsujino N, Funahashi H, Honda K, Guan JL, Wang QP, Tominaga M, Goto K, Shioda S, Sakurai T. Orexins activate histaminergic neurons via the orexin 2 receptor. Biochem Biophys Res Commun. 2002; 290:1237–1245. [PubMed: 11811995]

Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, Yanagisawa M, Sakurai T. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. Neuron. 2003; 38:701–713. [PubMed: 12797956]

Yoshida K, Mccormack S, Espana RA, Crocker A, Scammell TE. Afferents to the orexin neurons of the rat brain. J Comp Neurol. 2006; 494:845–861. [PubMed: 16374809]

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

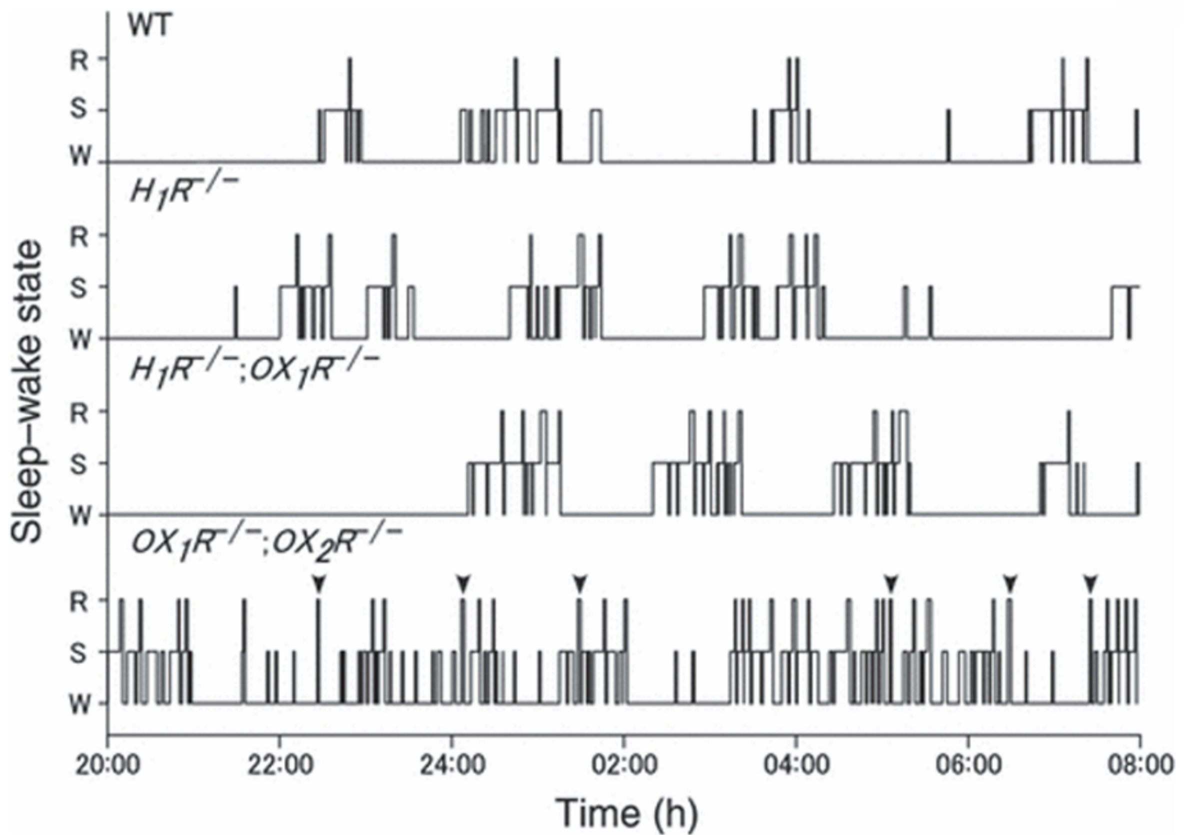

Figure 1.

Representative 12 h dark period (20:00 hours to 08:00 hours) hypnograms for wild type mice (WT), $H_1R^{-/-}$ mice, $H_1R^{-/-}$; $OX_1R^{-/-}$ mice and $OX_1R^{-/-}$;$OX_2R^{-/-}$ mice. The height of the horizontal line above baseline indicates the vigilance state of the mouse at the time. W, wakefulness; NR, non-rapid eye movement (REM) sleep; R, REM sleep. There were no significant differences in sleep/wake phenotypes between wild type mice, $H_1R^{-/-}$ mice, and $H_1R^{-/-}$;$OX_1R^{-/-}$ mice. In contrast, $OX_1R^{-/-}$;$OX_2R^{-/-}$ mice showed severe fragmentation of sleep/wake states, along with frequent direct transitions from wakefulness to REM sleep (mark with arrowhead). Hypnograms were obtained by simultaneous EEG/EMG recording as described previously (Chemelli *et al.* 1999).

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

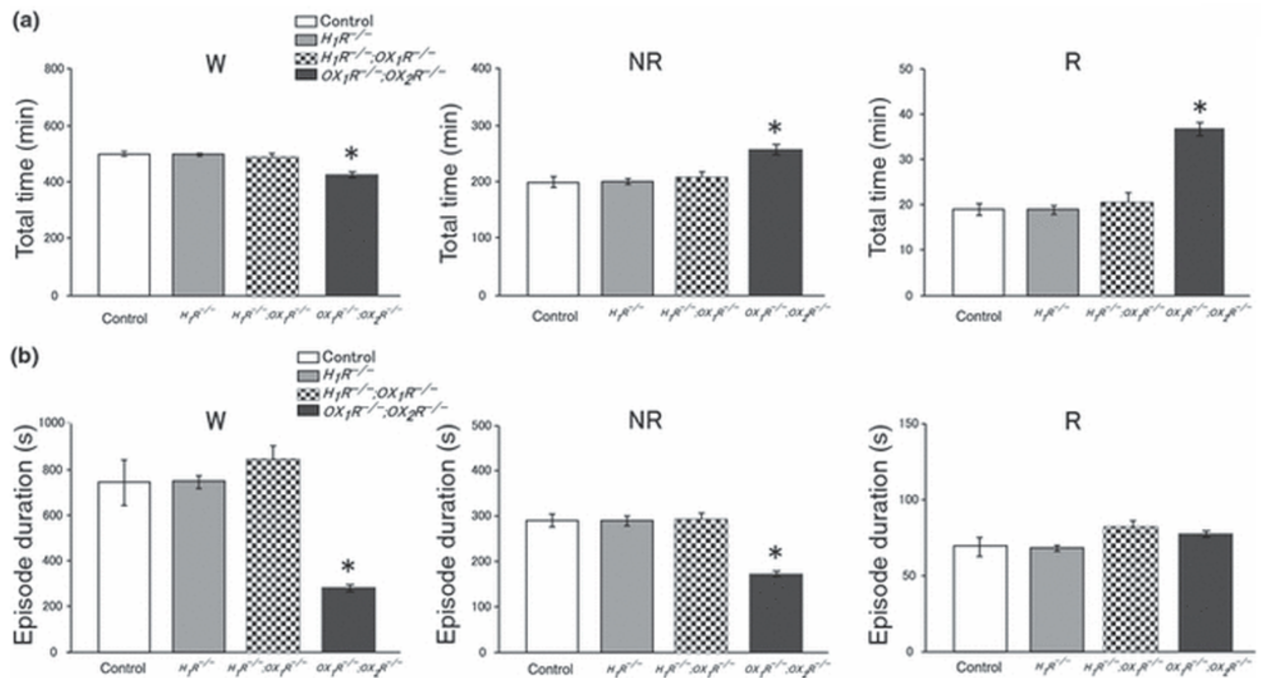

Figure 2.

(a) Total time (min, mean ± SEM) spent in each state in control mice (wild type) ($n = 5$), $H_1R^{-/-}$ mice ($n = 4$), $H_1R^{-/-}$; $OX_1R^{-/-}$ mice ($n = 4$) and $OX_1R^{-/-}$;$OX_2R^{-/-}$ mice ($n = 5$). W, awake; NR, non-rapid eye movement (REM) sleep; R, REM sleep. The graphs summarize the data recorded during the 12 h dark period. (B) Episode duration (s ± SEM) spent in each state in control mice, $H_1R^{-/-}$ mice, $H_1R^{-/-}$;$OX_1R^{-/-}$ mice and $OX_1R^{-/-}$;$OX_2R^{-/-}$ mice. W, awake; NR, non-REM sleep; R, REM sleep. $OX_1R^{-/-}$; $OX_2R^{-/-}$ mice show significantly shorter wakefulness and NREM sleep episodes during dark period. Mice with other genotypes did not show any abnormality in sleep/wake states. *$P < 0.001$. The graphs summarize the data recorded during the 12 h dark period.

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

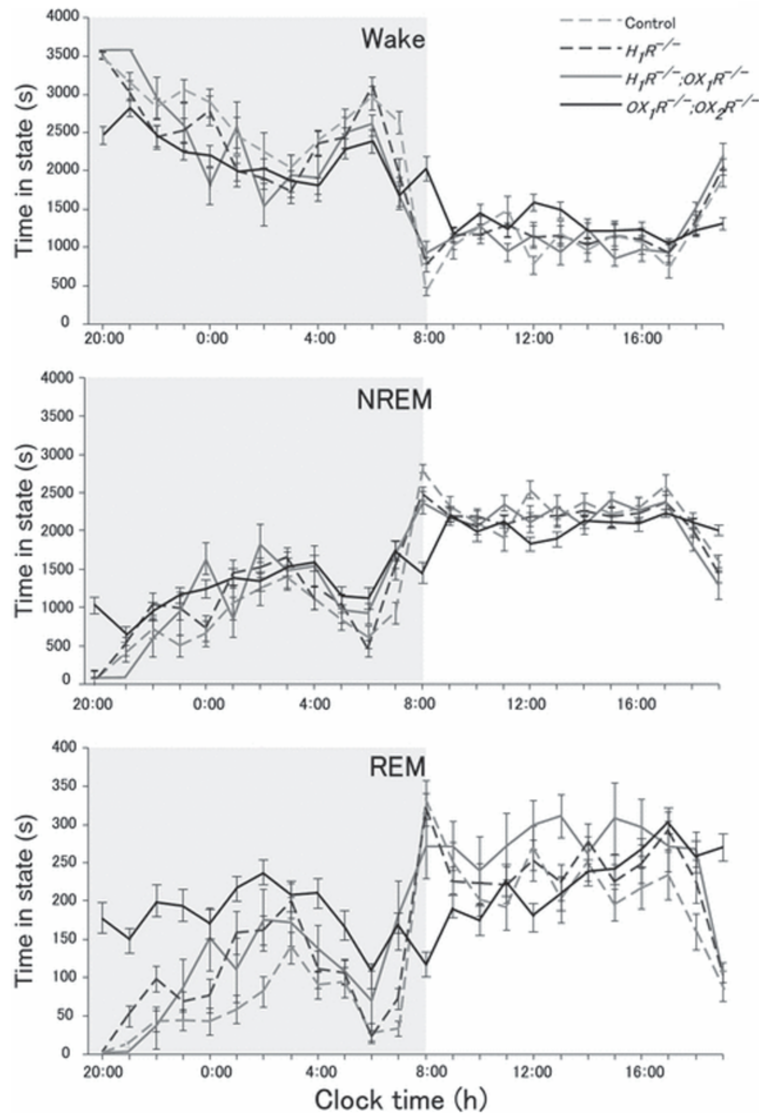

Figure 3.

Hourly analysis of sleep/wake amounts in control mice ($n = 5$), $H_1R^{-/-}$ mice ($n = 4$), $H_1R^{-/-};OX_1R^{-/-}$ mice ($n = 4$) and $OX_1R^{-/-};OX_2R^{-/-}$ mice ($n = 5$). The shaded areas represent the 12 h dark period. There are no significant differences between control mice, $H_1R^{-/-}$ mice, $H_1R^{-/-};OX_1R^{-/-}$ mice, while $OX_1R^{-/-};OX_2R^{-/-}$ mice show abnormal circadian distribution of rapid eye movement (REM) sleep.

HHMI Author Manuscript HHMI Author Manuscript HHMI Author Manuscript

Table 1

Total time spent in each state (min, mean ± SEM), episode duration (s, mean ± SEM), and rapid eye movement (REM) latency (min, mean ± SEM) over 24 h, itemized separately for light and dark periods

	REM sleep				Non-REM sleep				Awake			
	WT	$H_1R^{-/-}$	$H_1R^{-/-}/OX_1R^{-/-}$	$OX_1R^{-/-}/OX_2R^{-/-}$	WT	$H_1R^{-/-}$	$H_1R^{-/-}/OX_1R^{-/-}$	$OX_1R^{-/-}/OX_2R^{-/-}$	WT	$H_1R^{-/-}$	$H_1R^{-/-}/OX_1R^{-/-}$	$OX_1R^{-/-}/OX_2R^{-/-}$
24 h												
Total time (min)	65.8 ± 1.6	66.9 ± 2.4	70.0 ± 4.2	81.3 ± 0.9	636.1 ± 13.4	635.2 ± 6.6	639.5 ± 13.7	651.7 ± 12.0	735.1 ± 11.5	737.9 ± 7.0	726.2 ± 16.8	707.0 ± 12.7
Episode duration (s)	71.5 ± 3.2	70.0 ± 1.5	82.8 ± 2.4	71.48 ± 2.0	288.9 ± 16.2	287.3 ± 7.0	289.9 ± 9.5	216.4 ± 9.2	456.1 ± 107.9	452.7 ± 49.4	531.7 ± 69.7	247.2 ± 16.3*
REM latency (min)	368.3 ± 19.1	353.0 ± 9.9	364.1 ± 15.8	232.5 ± 49.6*								
REM counts	25.4 ± 2.1	25.8 ± 2.0	28.2 ± 3.3	32.8 ± 3.8								
Light period												
Total time (min)	46.8 ± 1.3	47.9 ± 1.6	49.4 ± 3.0	44.6 ± 1.3	435.7 ± 5.7	433.4 ± 4.9	430.5 ± 6.7	405.6 ± 3.2	233.4 ± 5.0	238.6 ± 5.5	235.8 ± 7.4	269.83 ± 3.2
Episode duration (s)	73.6 ± 3.0	71.8 ± 2.2	83.5 ± 2.7	66.9 ± 3.4	286.4 ± 31.0	284.0 ± 9.0	287.8 ± 9.6	239.4 ± 15.3	165.6 ± 18.3	155.8 ± 4.5	214.6 ± 13.0	163.4 ± 9.1
REM latency (s)	328.3 ± 18.7	323.1 ± 10.1	328.5 ± 12.9	282.2 ± 9.5								
REM counts	35.13 ± 2.3	37.1 ± 2.1	36.8 ± 4.7	37 ± 0.9								
Dark period												
Total time (min)	19.0 ± 1.2	19.0 ± 1.0	20.6 ± 2.1	36.7 ± 1.0*	200.4 ± 9.7	201.8 ± 5.4	209.0 ± 10.4	246 ± 7.3*	501.7 ± 8.1	499.2 ± 5.6	490.4 ± 12.0	437 ± 7.8*
Episode duration (s)	69.4 ± 6.0	68.2 ± 2.0	82.1 ± 4.2	76 ± 1.7	291.4 ± 14.6	290.4 ±10.8	292.1 ± 16.8	193 ± 8.2*	746.6 ± 99.5	749.5 ± 27.1	848.8 ± 57.5	331 ± 21.0*
REM latency (s)	403.2 ± 29.6	382.2 ± 13.7	399.7 ± 25.6	182 ± 7.3*								
REM counts	15.7 ± 1.2	16.7 ± 0.9	19.6 ± 3.2	29 ± 1.0*								

There were no significant differences between control mice, $H_1R^{-/-}$ mice and $H_1R^{-/-};OX_1R^{-/-}$ mice. $OX_1R^{-/-};OX_2R^{-/-}$ mice showed significantly shorter duration of wakefulness. REM latency is the average time of non-REM sleep duration before each REM sleep epoch. REM counts are numbers of REM sleep during each period.

* $P < 0.001$.