J Mol Neurosci (2011) 43:155–161
DOI 10.1007/s12031-010-9437-7

Ectopic Overexpression of Orexin Alters Sleep/Wakefulness States and Muscle Tone Regulation during REM Sleep in Mice

Jon T. Willie · Hitomi Takahira · Megumi Shibahara · Junko Hara · Mika Nomiyama · Masashi Yanagisawa · Takeshi Sakurai

Received: 31 May 2010 / Accepted: 2 August 2010 / Published online: 14 August 2010

Abstract Orexins (also called hypocretins), which are neuropeptides exclusively expressed by a population of neurons specifically localized in the lateral hypothalamic area, are critically implicated in the regulation of sleep/wake states. Orexin deficiency results in narcoleptic phenotype in rodents, dogs, and humans, suggesting that orexins are important for maintaining consolidated wakefulness states. However, the physiological effect of constitutive increased orexinergic transmission tone, which might be important for understanding the effects of orexin agonists that are promising candidates for therapeutic agents of narcolepsy, has not been fully characterized. We report here the sleep/wakefulness abnormalities in transgenic mice that exhibit widespread overexpression of a rat *prepro-orexin* transgene driven by a β-actin/cytomegalovirus hybrid promoter (*CAG/orexin* transgenic mice). *CAG/orexin* mice exhibit sleep abnormalities with fragmentation of non-rapid eye movement (REM) sleep episode and a reduction in REM sleep. Non-REM sleep was frequently disturbed by short episodes of wakefulness. EEG/EMG studies also reveal incomplete REM sleep atonia with abnormal myoclonic activity during this sleep stage. These results suggest that endogenous orexinergic activity should be appropriately regulated for normal maintenance of sleep states. Orexinergic transmission should be activated during wakefulness, while it should be inactivated or decreased during sleep state to maintain appropriate vigilance states.

Keywords Orexin · Hypocretin · Sleep · Wakefulness · Overexpression · Transgenic

This manuscript is related to the GPCR2010 symposium.

Electronic supplementary material The online version of this article (doi:10.1007/s12031-010-9437-7) contains supplementary material, which is available to authorized users.

J. T. Willie · M. Yanagisawa
Howard Hughes Medical Institute and Department of Molecular Genetics,
University of Texas Southwestern Medical Center at Dallas,
Dallas, TX 75390-9050, USA

H. Takahira · M. Shibahara · J. Hara · T. Sakurai
Department of Pharmacology, Institute of Basic Medical Sciences,
University of Tsukuba,
Ibaraki 305-8575, Japan

M. Nomiyama · M. Yanagisawa · T. Sakurai
ERATO Yanagisawa Orphan Receptor Project,
Japan Science and Technology Corporation,
Tokyo 135-0064, Japan

J. Hara · T. Sakurai
Department of Molecular Neuroscience and Integrative Physiology,
Faculty of Medicine, Kanazawa University,
Kanazawa,
Ishikawa 920-8640, Japan

T. Sakurai (✉)
Department of Molecular Neuroscience and Integrative Physiology, Faculty of Medicine,
Kanazawa University,
13-1 Takaramachi, Kanazawa-shi,
Ishikawa 920-8640, Japan
e-mail: tsakurai@med.kanazawa-u.ac.jp

Introduction

Orexin-A and orexin-B (also called hypocretin-1 and hypocretin-2) are neuropeptides proteolytically produced from a common precursor that are expressed in a specific population of neurons in the lateral hypothalamic area (Sakurai et al. 1998). Orexin-producing neurons (orexin neurons) send axons throughout the brain with especially dense projections to monoaminergic and cholinergic nuclei in the brainstem, which are regions implicated in the regulation of sleep/wakefulness. The finding of orexin deficiency in narcolepsy patients suggests that this hypothalamic neuropeptide plays a crucial role in regulating sleep/wake states (Sakurai 2007). The implication of orexin in narcolepsy suggests the importance of orexin in the normal regulation of vigilance states.

Because activation of orexin receptors by means of orexin agonists or gene therapy is a promising avenue towards therapeutic trials for narcolepsy and potentially other sleep disorders, it is essential to identify any alteration in the sleep/wake architecture as a result of constitutive increase in orexinergic tone. However, the sleep/wake phenotype resulting from constitutive increased orexinergic tone in the brain has not been thoroughly examined. Although we previously reported some of the pharmacological effects of continuous infusion of orexin-A into the lateral ventricle of rats, we only reported the metabolic parameters (Yamanaka et al. 1999). Effects of orexin administration on vigilance parameters have been examined thus far only by bolus administration (Huang et al. 2001; Piper et al. 2000). To examine the effects of chronically increased orexinergic tone in mice, we made transgenic mouse lines that exhibit widespread constitutive expression of a rat *prepro-orexin* transgene driven by a β-actin/cytomegalovirus hybrid promoter (*CAG/orexin* transgenic mice; Mieda et al. 2004). We previously reported that this transgene completely prevented cataplectic arrests and other abnormalities of rapid eye movement (REM) sleep of *orexin/ataxin-3* narcoleptic mice in which endogenous orexin neurons are absent (Mieda et al. 2004). We also reported that these mice are resistant to fat diet-induced obesity and show increased insulin insensitivity by promoting energy expenditure (Funato et al. 2009). However, sleep abnormality of these mice have not been reported in detail so far. Here, we describe the sleep/wake phenotype of the *CAG/orexin* transgenic mice in detail. These mice exhibit sleep abnormalities with fragmentation of non-REM sleep and a specific reduction in REM sleep. EEG/EMG studies also reveal incomplete REM sleep atonia with abnormal myoclonic activity during this sleep stage. These results suggest that *CAG/orexin* mice have an inability to maintain sleep states and reveal the physiological importance of inhibitory controls of the orexin system in the regulation of sleep/wakefulness states and muscle tone especially during REM sleep. The sleep abnormalities in these mice with orexin overexpression suggests that relatively short half-life is desirable for orexinergic agonists developed for the treatment of narcolepsy or other sleep disorders as unwanted abnormalities in sleep or muscle tone may occur with prolonged increased orexinergic tone. Gene therapy approach with constitutive expression of orexin will cause sleep fragmentation. Methods with proper regulation of orexin expression according to vigilance states will be required for such an approach.

Materials and Methods

Animal Care

All experimental procedures involving animals were approved by the University of Tsukuba Animal Care and Use Committee, the Kanazawa University Animal Care and Use Committee, and the Institutional Animal Care and Research Advisory Committee of the University of Texas Southwestern Medical Center at Dallas and were in accordance with the NIH guidelines.

CAG/orexin Transgenic Mice

To distinguish endogenous mouse orexin gene and transgene, we used rat *prepro-orexin* cDNA (585 bp) as a transgene, which was inserted between two *Eco*RI sites of pCAGGS (Niwa et al. 1991). The transgene was excised by digesting with *Sal*I and *Pst*I, gel-purified, and then injected into pronuclei of fertilized eggs obtained from DBF1 mice. We obtained six transgenic lines. Copy numbers of the transgene in these lines were 1, 3, 3, 3, 12, and 12 as determined by genomic Southern hybridization analysis (data not shown). In the present study, we used the lines L2 and L62 for analyses, which have copy numbers of 3 and 12, respectively (Table 1). All experiments utilized male and female mice from crosses in which hemizygous transgenic mice were bred to wild-type C57BL/6J mice. Offspring (DBA1 backcrossed to C57BL/6J, N5–N7) were weaned at 21 days and genotyped by PCR using the following primers: 5′-GTTGTGCTGTCTCATCATTTTGGC-3′ and 5′-AACGTCTTTATTGCCTAGGGACCG-3′.

Expression of Orexin in the *CAG/orexin* Mice

Expression levels of *orexin* mRNA and peptide levels in the brains of transgenic lines were examined by Northern blot hybridization analysis and radioimmunoassay, respectively. For Northern blot hybridization, total RNA from the brains of 4-week-old transgenic mice were isolated, separated by electrophoresis on a 1.0% agarose/formaldehyde gel,

Table 1 Expression levels of orexin mRNA and peptide in six *CAG/orexin* transgenic lines

Line	Copy numbers	Orexin-A content (ng/g brain)	Orexin-B content (ng/g brain)	*Prepro-orexin* mRNA (relative to control)
Control	0	9.3 (1.0)	14.0 (1.0)	1.0
L25	1	37.2 (4.0)	76.1 (5.4)	1.2
L13	3	111.8 (12.0)	214.0 (15.2)	1.9
L28	3	145.2 (15.6)	319.0 (22.7)	3.3
L2	3	174.0 (18.7)	427.0 (30.4)	3.8
L64	12	264.1 (28.4)	971.7 (69.4)	7.9
L62	12	276.2 (29.7)	1,104.4 (78.6)	7.2

Peptide levels were determined by radioimmunoassay and *prepro-orexin* mRNA levels were determined by Northern blot hybridization in brains from each line

blotted onto nylon membranes, and hybridized with a rat $\alpha[^{32}P]$-labeled *prepro-orexin* cDNA probe. Membranes were subjected to autoradiography and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. Relative content of *prepro-orexin* mRNA was determined by densitometry and calculated as a ratio of orexin/GAPDH. For radioimmunoassay, brains from mice 4 weeks of age for each line were homogenized with a Polytron in 70% (*v*/*v*) acetone, 1 M acetic acid, and 20 mM HCl. The homogenate was centrifuged at 20,000×*g* for 30 min. The resultant supernatant was collected and extracted twice with diethyl ether. The supernatant was loaded onto SepPak C18 cartridges (Waters), which were pre-equilibrated with 0.1% (*v*/*v*) trifluoroacetic acid (TFA). Cartridges were washed with 5% CH_3CN/0.1% TFA and then eluted with 50% CH_3CN/0.1% TFA. The eluate was lyophilized and subjected to radioimmunoassay using commercially available [^{125}I]RIA kits (Phoenix Pharmaceuticals).

Sleep Studies

EEG/EMG monitoring and analysis was performed as previously described (Chemelli et al. 1999; Hara et al. 2001). For quantifying abnormal motor activity during REM sleep, all REM sleep events during a 24-h period were scored (three animals per genotype). Phasic muscle activity during REM sleep was defined as an EMG deflection >50% above the baseline. A tonic activity event during REM sleep was scored when a series of deflections lasted for longer than 0.3 s. Statistical comparisons were performed by ANOVA by post hoc analysis of significance by Fisher's protected least significant difference test.

Histological Analysis

Mice were anesthetized by intraperitoneal injection of sodium pentobarbital and perfused via the heart with phosphate-buffered saline (PBS), followed by 0.1 M phosphate buffer containing 4% paraformaldehyde. The whole brain was post-fixed in 4% paraformaldehyde in PBS overnight at 4°C. Cryostat sections (40-μm thick) were preincubated with a solution of 1% bovine serum albumin diluted in 0.3% Triton X-100 in PBS for 1 h and then incubated overnight at 4°C with goat anti-ChAT antibody (Chemicon; 1:200) and rabbit anti-orexin antiserum (1:1,000; Nambu et al. 1999) diluted in a fresh hybridization solution. After washing three times in 0.3% Triton X-100 in PBS, the sections were incubated with Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Molecular Probes; 1:250) for 90 min at room temperature and then washed three times in 0.3% Triton X-100 in PBS. Tissues were subsequently incubated with either an Alexa Fluor 594-conjugated donkey anti-goat IgG or goat anti-mouse IgG (Molecular Probes; 1:250) (Cappel) for 90 min at room temperature. The sections were then washed three times in 0.3% Triton X-100 in PBS and mounted onto glass slides and coverslipped. Slides were examined under a fluorescence microscope. Specificity of all antibodies was confirmed using a preabsorption with each respective substance and observing no signal.

Results

Independent Lines of *CAG/orexin* Transgenic Mice

Immunohistochemical analysis of the brains of one of the transgenic lines, namely, L2, was previously reported (Mieda et al. 2004). Patterns of orexin expression in the brains of each line were similar among each line (data not shown). The absolute amounts of brain *orexin* expression (as determined by Northern blots) and peptide levels of orexin-A and orexin-B (as determined by orexin-A RIAs) varied with each transgenic line (Table 1).

In this study, we utilized two lines, L2 and L62, which show moderate and high expression levels, respectively (Table 1). Because a subtle gene–dosage effect was observed in parameters of vigilance states between the L2 and L62 lines, the data are reported separately for each line.

Sleep/Wakefulness Abnormalities in *CAG/orexin* Mice

To examine the effects of orexin overexpression on spontaneous sleep/wake patterns, we monitored the EEG/EMG of

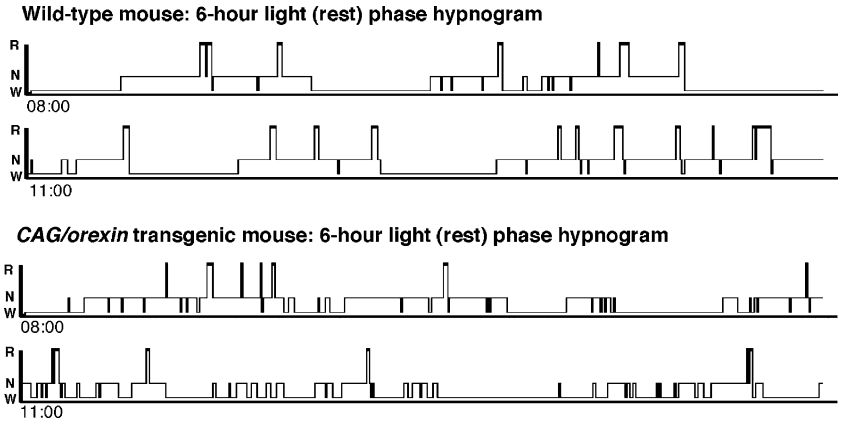

Fig. 1 Illustrative hypnograms from wild-type and *CAG/orexin* transgenic mice. Sleep/wake patterns (*W* wakefulness, *N* non-REM sleep, *R* REM sleep) generated from concatenating EEG/EMG stage scores over the first 6 h of the light phase are shown. Note the increased fragmentation of non-REM sleep and reduced amounts and durations of REM sleep in transgenic mouse. The hypnograms of the *CAG/orexin* mouse shown is from line L2

mice from two independent lines (L2 and L62) of *CAG/orexin* transgenic mice and their wild-type littermates. Hypnograms from individual mice of each genotype during the light and dark phases illustrate the behavior of these animals (Fig. 1). Compared with wild-type controls, *CAG/orexin* mice exhibited evidence of disturbed sleep especially during the light phase (rest period). In particular, *CAG/orexin* mice have increased fragmentation of sleep patterns during the light phase when normal mouse behavior is predominantly sleep. Increases in the number of wakefulness episodes and decreases in the duration of non-REM and REM sleep support this observation (Fig. 2). Most notably, normal

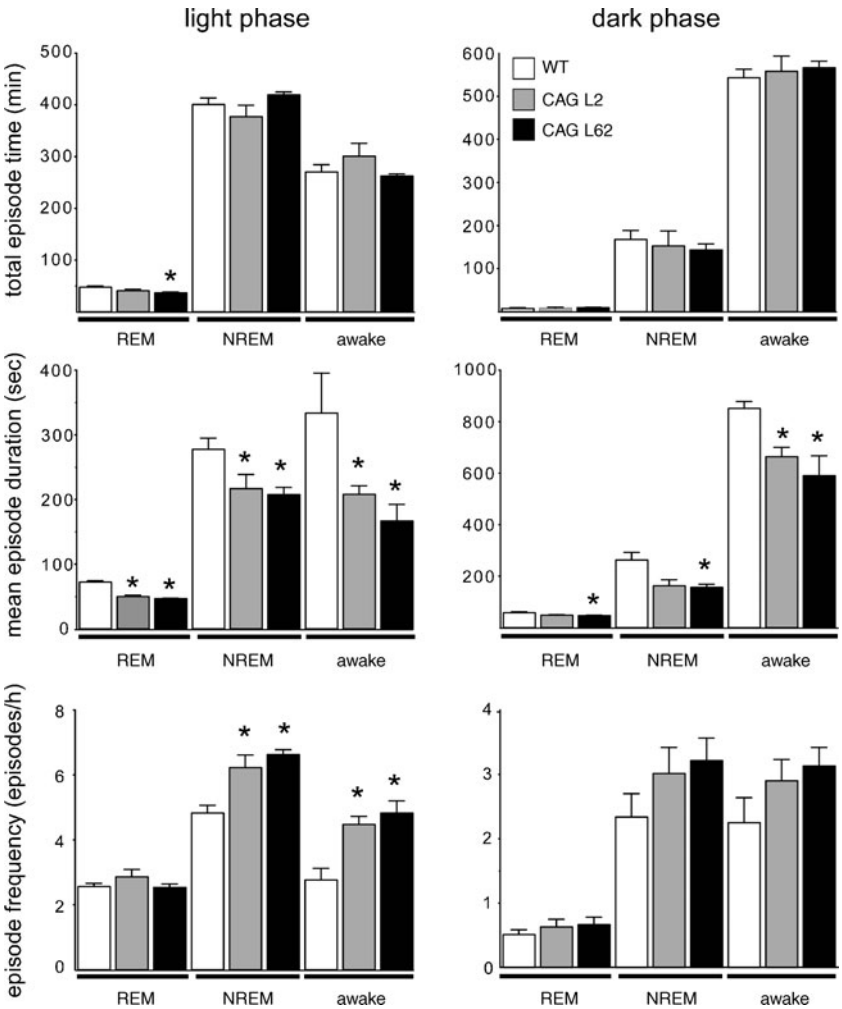

Fig. 2 Sleep/wakefulness parameters recorded from two *CAG/orexin* transgenic lines and wild-type controls. Total time spent in each state (in minutes; **a**), episode duration (in seconds; **b**), and episode frequency (per hour; **c**) over light and dark periods (*left* and *right panels*, respectively) are shown. Statistical analysis was tested by repeated-measures ANOVA followed by Tukey post hoc test. *$p<0.05$, significant differences of transgenic groups compared with the wild-type group

patterns of REM sleep are profoundly disturbed in transgenic mice during both phases. *CAG/orexin* mice showed significant reduction in the amount of REM sleep during the light phase and significantly reduced duration of REM sleep time across light and dark phases (Fig. 2). Additionally, *CAG/orexin* mice exhibited reduced REM latencies: REM latencies of wild-type mice, CAG L2 mice, and CAG L62 mice were 9.05+0.64 min, 6.17+0.61 min ($p<0.05$), and 6.19+0.17 min ($p<0.05$), respectively, for the light period and 8.33+0.63 min, 5.42+0.92 min ($p<0.05$), and 4.92+0.53 min ($p<0.05$), respectively, for the dark period. These observations strongly suggest that chronically increased orexinergic tone disrupts normal sleep architecture.

Abnormalities of Muscle Tonus in *CAG/orexin* Mice during REM Sleep

Close observation of the structural features of EEG/EMG patterns reveals other abnormalities in *CAG/orexin* mice as well. The onset of REM sleep in normal wild-type mice is characterized by REM sleep atonia, a further reduction of postural muscle tone compared with that preceding non-REM sleep. Although twitching of peripheral muscles (e.g., hands, feet, facial musculature) often occurs during REM sleep, postural muscles remain virtually quiet. In contrast, *CAG/orexin* mice exhibited sporadic increases in postural muscle tone with the onset of REM sleep. A striking increase in the frequency and amplitude of phasic twitches recorded from nuchal muscles was also observed ($p=0.05$; Fig. 3a, b).

Interestingly, abnormalities of atonia in REM sleep have reportedly been associated with narcolepsy in humans. Therefore, for comparison, we also examined narcoleptic *orexin/ataxin-3* transgenic mice (Hara et al. 2001) on a similar genetic background as the *CAG/orexin* mice. Notably, *orexin/ataxin-3* mice also exhibited a mild increase in phasic muscle activity during REM sleep, although it was of an intermediate frequency relative to wild-type and *CAG/orexin* transgenic mice (Fig. 3b). On the other hand, *orexin/ataxin-3* mice showed normal frequencies of tonic muscle activity during REM sleep.

These observations suggest that instability of the muscle tone regulatory system in REM sleep is disrupted in both orexin-deficient and overexpressing mice, suggesting that orexins both positively and negatively affect muscle tone during REM sleep.

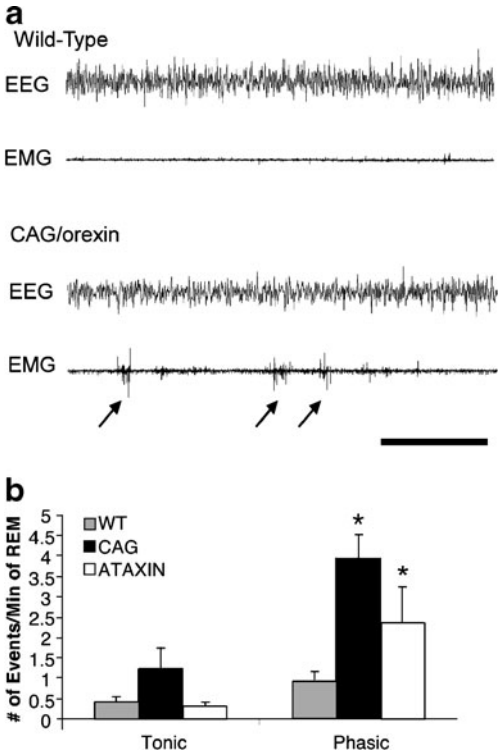

Fig. 3 Abnormalities of REM sleep atonia in *CAG/orexin* transgenic mice. **a** Illustrative EEG/EMG traces reflect REM sleep in wild-type and transgenic mice. Note increased postural tone and increased phasic activity (*arrows*) evident in the EMG of the transgenic mouse. **b** Frequencies of tonic and phasic activities in wild-type, *CAG/orexin*, and *orexin/ataxin-3* transgenic mice during REM sleep. Note high frequencies of abnormal tone and phasic activity in *CAG/orexin* mice as well as a milder elevation of phasic activity in *orexin/ataxin-3* transgenic mice

Discussion

Our results demonstrate that constitutive and ectopic increase of orexinergic transmission results in complex alterations of sleep/wakefulness states. Primarily, orexin overexpression induced an inability to maintain sleep states as evidenced by increased number of wakefulness and non-REM sleep episodes across light and dark phases and a selective decrease in the duration of non-REM sleep episodes during the light phase. These findings contrast with similar studies conducted on mice with orexin-deficient narcolepsy in which animals are primarily unable to maintain wakefulness states during the dark phase (Chemelli et al. 1999; Hara et al. 2001). Strong disruption of normal sleep states was also evident in the *CAG/orexin* transgenic mice (Figs. 1 and 2). While narcoleptic orexin-deficient mice display frequent and abnormal intrusions of REM sleep-related phenomena into wakefulness, *CAG/orexin* transgenic mice displayed a significant decrease in the amount of REM sleep during the light phase and reduced REM sleep duration across both light and dark phases. These findings are consistent with a role of orexin in the inhibition of REM sleep. However, reduced REM latency and REM intervals in the *CAG/orexin* transgenic mice also suggests that maintenance of non-REM sleep is also disturbed by intrusion of REM sleep as well as wakefulness.

The finding that orexin imbalance also disrupted mechanisms of REM sleep atonia may have particular

clinical relevance for disorders of abnormal muscle activity and myoclonus during sleep such as restless legs syndrome (RLS) or REM sleep behavior disorder (Schenck and Mahowald 2002). Indeed, one study indicated that early-onset familial forms of RLS are associated with higher levels of orexin-A in the cerebrospinal fluid (Allen et al. 2002). In this study, we identified increases in phasic muscular activity during REM sleep in both orexin over-expressing mice as well as in orexin neuron-ablated *orexin/ataxin-3* mice. A potentially complex role of orexins in such processes is also indicated by orexin microinjection studies in cats that suggest that REM sleep-related muscle atonia can be facilitated or inhibited depending on the site of injection in brainstem areas that have been previously implicated in the control of REM sleep atonia (Mileykovskiy et al. 2002). Muscle tone during REM sleep is inhibited via an activation of cholinergic neurons in the pedunculopontine/laterodorsal tegmental nucleus (PPN/LDT). These cholinergic neurons activate glycinergic cells that send inhibitory projections to motor neurons of postural muscles. Orexin neurons send abundant projections to the PPN/LDT (Supplementary Figure 1). Therefore, orexins might regulate the activity of these cholinergic cells, and this mechanism might be important in the pathophysiology of cataplexy in narcolepsy. A study suggests that orexins inhibit cholinergic neurons in the PPN via an activation of local GABAergic interneurons, although orexins have also been shown to evoke direct excitatory effect on the cholinergic neurons in cats (Takakusaki et al. 2005). Constitutive elevation of orexinergic tone in the brain of the *CAG/orexin* mice might disturb the normal muscle tone regulatory mechanism in LDT/PPN regions during REM sleep. Interestingly, *orexin/ataxin-3* mice, in which orexin neurons are genetically ablated, also show a mild increase in phasic muscle activity during REM sleep. These observations that either an increase or decrease in orexinergic tone increases phasic muscle activity during REM sleep suggest an intriguing possibility. Although recent studies show that orexin neurons are active during wakefulness and silent during both non-REM and REM sleep (Lee et al. 2005; Mileykovskiy et al. 2005), some populations of orexin neurons might send direct excitatory projections to cholinergic cells and could be active during REM sleep to keep muscle tone silent. On the other hand, a different population of orexin neurons might send inhibitory signals to these cholinergic cells via an activation of GABA interneurons and/or monoaminergic neurons.

The sleep/wake phenotype of the *CAG/orexin* mice is totally different from that of mice or rats with pharmacological administration of orexins, which is characterized by prolonged wakefulness. The difference might possibly stem from chronic alterations by plasticity of neurons which express orexin receptors.

Our present findings suggest that the activity of orexin neurons should be appropriately decreased or turned off during sleep to maintain normal sleep. This regulation might possibly be through GABAergic inhibition by the innervation from the sleep-active neurons in the preoptic areas (Sakurai et al. 2005; Yoshida et al. 2006). Our present results also suggest that, if orexinergic agonists are developed as a therapeutic agent for narcolepsy, a relatively short half-life is required in order to avoid any possible unwanted sleep or muscle tone disturbances. Finally, careful regulation of orexinergic tone could potentially be of therapeutic benefit not only for narcoleptic patients but also for patients suffering from abnormal muscle activity and myoclonus during sleep.

Acknowledgements This study was supported in part by a grant-in-aid for scientific research from the 21st Century COE Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, a grant for anorexia nervosa research from the Japanese Ministry of Health, Labour and Welfare, Uehara Memorial Foundation and research grants from the ERATO/JST. M.Y. is an investigator of the Howard Hughes Medical Institute. J.T.W. is a joint fellow of the DCMB and MST programs of University of Texas Southwestern Medical Center at Dallas.

References

Allen RP, Mignot E, Ripley B, Nishino S, Earley CJ (2002) Increased CSF hypocretin-1 (orexin-A) in restless legs syndrome. Neurology 59:639–641

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell TE et al (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98:437–451

Funato H, Tsai A, Willie JT, Kisanuki Y, Williams SC et al (2009) Enhanced orexin signaling prevents diet-induced obesity and improves leptin sensitivity. Cell Metab 9:64–76

Hara J, Beuckmann CT, Nambu T, Willie JT, Chemelli RM et al (2001) Genetic ablation of orexin neurons in mice results in narcolepsy, hypophagia, and obesity. Neuron 30:345–354

Huang ZL, Qu WM, Li WD, Mochizuki T, Eguchi N et al (2001) Arousal effect of orexin A depends on activation of the histaminergic system. Proc Natl Acad Sci USA 98:9965–9970

Lee MG, Hassani OK, Jones BE (2005) Discharge of identified orexin/hypocretin neurons across the sleep–waking cycle. J Neurosci 25:6716–6720

Mieda M, Willie JT, Hara J, Sinton CM, Sakurai T, Yanagisawa M (2004) Orexin peptides prevent cataplexy and improve wakefulness in an orexin neuron-ablated model of narcolepsy in mice. Proc Natl Acad Sci USA 101:4649–4654

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2002) Muscle tone facilitation and inhibition after orexin-a (hypocretin-1) microinjections into the medial medulla. J Neurophysiol 87:2480–2489

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798

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

Niwa H, Yamamura K, Miyazaki J (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108:193–199

Piper DC, Upton N, Smith MI, Hunter AJ (2000) The novel brain neuropeptide, orexin-A, modulates the sleep–wake cycle of rats. Eur J Neurosci 12:726–730

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

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM et al (1998) Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell 92:573–585

Sakurai T, Nagata R, Yamanaka A, Kawamura H, Tsujino N et al (2005) Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. Neuron 46:297–308

Schenck CH, Mahowald MW (2002) REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP. Sleep 25:120–138

Takakusaki K, Takahashi K, Saitoh K, Harada H, Okumura T et al (2005) Orexinergic projections to the midbrain mediate alternation of emotional behavioral states from locomotion to cataplexy. J Physiol 568(Pt 3):1003–1020

Yamanaka A, Sakurai T, Katsumoto T, Yanagisawa M, Goto K (1999) Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res 849:248–252

Yoshida K, McCormack S, Espana RA, Crocker A, Scammell TE (2006) Afferents to the orexin neurons of the rat brain. J Comp Neurol 494:845–861