A Quartet Neural System Model Orchestrating Sleep and Wakefulness Mechanisms

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INTRODUCTION

Our knowledge of the mechanism regulating sleep and waking states is continuously advancing. Examples of such advancements include findings of the involvement of neurons in the ventrolateral preoptic hypothalamic area (VLPO) and the median preoptic nucleus (MnPN) in the regulation of non-rapid eye movement (NREM) sleep as well as rapid eye movement (REM) sleep (Sherin et al. 1996; Suntsova et al. 2002). The interactions of these neuron groups with those regulating wakefulness and REM sleep in the perifornical/posterior hypothalamic nuclei and the brain stem (BS) have been suggested to underlie the physiological mechanisms for maintenance and alternation of sleep and wakefulness (McInty and Szymusiak 2003; Saper et al. 2001). However, it is not yet known how sleep and waking states are maintained and changed by the combination of these nuclei.

Mathematical models have improved our understanding of the neural mechanisms of sleep-wake control. Among these models, reciprocal interaction models have portrayed the fundamental mechanism of the REM-NREM cycle, where the cholinergic and aminergic neuron groups are hypothesized to behave like a prey–predator system (McCarley and Hobson 1975; McCarley and Massaquoi 1986a,b). This model was subsequently extended conceptually (McCarley et al. 1995; Pace-Schott and Hobson 2002). Based on recent advancements in physiological knowledge, Saper proposed the conceptual flip-flop model of sleep-wakefulness regulation involving the VLPO, the perifornical hypothalamic orexin (hypocretin) neurons, the tuberomammillary nucleus (TMN), and the locus coeruleus (LC)/dorsal raphe nucleus (DR) (Saper et al. 2001). Despite this extension, an integrated model that can reproduce the patterns of change among NREM sleep, REM sleep, and wakefulness has yet to be mathematically described. How the flip-flop is flipped is not yet quantitatively understood. We previously constructed a model of the REM-NREM-wakefulness cycle by coupling formal neuron models and were able to reproduce the results of pharmacological experiments and narcoleptic behavior (Nakao et al. 1999). However, this model did not incorporate very recent findings such as those related to the VLPO, MnPN, and orexin neurons.

In this paper, we develop a model of sleep-wakefulness regulation in the rat that includes a neural regulator quartet of NREM sleep, REM sleep, wakefulness, and a coregulator of REM sleep and wakefulness. In constructing this model, we incorporated the findings of our extensive recordings of neuronal activities in the brainstem and hypothalamic areas across sleep and wakefulness states and also considered the physiological findings accumulated thus far. The physiological reality of the model structure is assessed by its ability to simulate the results of pharmacological experiments and the human sleep-wake cycle without being changed significantly. The results of the modeling suggest that the regulation of sleep and wakefulness is realized by well-orchestrated interactions among the aforementioned quartet of neural groups distributed in the hypothalamus and the BS with the aid of sleep-promoting substances.

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Summary of physiological knowledge

In this section, the physiological understanding of the neural groups involved in changing and maintaining sleep-wake states is summarized. Although the following description includes hypotheses that have not yet been proven, their correctness is tentatively assumed here for the purposes of modeling. These neuron groups are categorized mainly by their discharge patterns, which alternate depending on sleep-waking states, i.e., REM-ON, WA, N-R, and W-R. The REM-ON and WA neurons, respectively, exhibit increased and reduced activities exclusively in REM sleep. The N-R neurons are activated in NREM sleep and REM sleep, but not during wakefulness. The W-R neurons exhibit higher activity during wakefulness and REM sleep than during NREM sleep. Thus N-R and WA correspond to so-called “sleep-active” and “REM-off,” respectively. Figure 1 schematically shows the localizations of each neuron group (Datta 1995; Jones 2000; McCarley et al. 1995; McGinty and Szymusiak 2003; Sakai and Crochet 2003; Steriade and Hobson 1976). In addition to these depicted neurons, there are neurons exhibiting more phasic activity patterns, which might be involved in generation of sleep-related phasic events rather than in regulation of sleep and wakefulness (Datta 1995). Therefore this type of neuron will not be treated here.

In the following, for the REM-ON, WA, N-R, and W-R neurons mentioned above, putative functions are explained in each anatomical region together with their neurotransmitters and anatomical connections if available. Abbreviated names of neurotransmitters are in parenthesis next to the name of the neuron group, i.e., ACh, acetylcholine; Glu, glutamine; NA, noradrenaline; 5HT, serotonin; Orx, orexin; HA, histamine. Tentatively “Glu” is used to represent “putatively glutamnergic” in this paper, although the transmitter has not been precisely identified. Accordingly, each neuron is denoted by “nucleus or region”:“discharge type” (neurotransmitter). BS:REM-ON(ACH). Neurons of this type are distributed in the laterodorsal tegmental nucleus (LDT), the pedunculopontine tegmental nucleus (PPT), the peri-LCo, and the nucleus reticularis magnocellularis (NMC) in the medulla (Datta and Siwek 2002; El Mansari et al. 1989; Kayama et al. 1992; Sakai et al. 2001; Steriade et al. 1990). They are excited by REM-ON(Glu) neurons (Sakai and Koyama 1996), and inhibited by WA(5HT) neurons (Koyama and Kayama 1993; Leonard and Llinás 1994; Thakker et al. 1998). However, the responsiveness of a REM-ON(ACh) neuron in the PPT to monoaminergic inputs may differ from the LDT (Datta et al. 2003; Sanford et al. 1998). The BS:REM-ON(ACh) neurons have ascending projections, which are involved in generating phasic and tonic events during REM sleep such as rapid eye movements, PGO ( ponto-genulate-occipital) waves, and desynchronization of the EEG (Datta 2002; Datta et al. 2002; Onoe and Sakai 1995; Shouse and Siegel 1992).

BS:REM-ON(GLU). Neurons of this type are mostly distributed in the peri-LCo. This group of neurons is excited by the REM-ON(ACh) and W-R(ACh) neurons and inhibited by the WA(NA) and N-R(GABA) neurons in the preoptic region (POA) (Lu et al. 2002). They have descending projections, which are involved in muscle atonia during REM sleep (Sakai and Koyama 1996; Shouse and Siegel 1992). Mutual excitation between the REM-ON (ACh) and REM-ON (Glu) neurons is essential for generating REM sleep (Sakai and Crochet 2003).

BS:REM-ON(GABA). Neurons in this group are located in the mesopontine tegmental area (Maloney et al. 1999). One source of excitation of these neurons is the REM-ON (Glu) neurons. This group of neurons inhibits various WA neurons (Gervasoni et al. 1998; Nitz and Siegel 1996, 1997a,b). Nevertheless, there is no direct evidence that REM-ON(GABA) neurons inhibit WA neurons. However, c-fos expression, GABA release, and the existence of noncholinergic REM-on neurons all suggest the possibility of existence of REM-ON(GABA) neurons (Maloney et al. 1999; Nitz and Siegel 1996; Shouse and Siegel 1992).

LC:WA(NA). Neurons in this group are located in the LC (Aston-Jones and Bloom 1981; Cooper et al. 1996). They are excited by the WA(Orx) and W-R(ACh) neurons (Horvath et al. 1999; Koyama and Kayama 1993) and the TMN:WA(NA) neurons (Sakai 1991). They are inhibited by the REM-ON(GABA) and DR:WA(SHT) neurons and the N-R(GABA) neurons in the POA (Bobker and Williams 1989; Gallopin et al. 2000; Nitz and Siegel 1997b; Sakai 1991; Sherin et al. 1998). They function as a system permitting REM sleep by suppressing the REM-ON neurons (Hobson et al. 1975; Sakai and Koyama 1996).

DR:WA(SHT). Neurons of this type are located in the dorsal raphe nucleus (DR) (McGinty and Harper 1976). They are excited by the WA(Orx) (Brown et al. 2001; Takahashi et al. 2005) and other monoaminergic neurons such as the TMN:WA(NA) and LC:WA(NA) neurons (Sakai and Crochet 2000). They are inhibited by the REM-ON(GABA) and VLPO:N-R(GABA) neurons (Gervasoni et al. 2000; Nitz and Siegel 1997a; Saper et al. 2001; Sherin et al. 1998).

FIG. 1. Locations of neuronal groups involved in the regulation of sleep and wakefulness and state dependency of their discharge patterns (Datta 1995; McCarley et al. 1995; McGinty and Szymusiak 2003; Sakai and Crochet 2003; Steriade and Hobson 1976). Here, the neurons that have state-dependent but phasic activity patterns are not included (Datta 1995). VLPO, ventrolateral preoptic hypothalamic area; MnPN, median preoptic nucleus; TMN, tuberomammillary nucleus; LC, locus coeruleus; DR, dorsal raphe nucleus; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus; peri-LCo, peri-locus coeruleus α; NMC, nucleus reticularis magnocellularis; BF, basal forebrain.

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Lateral and posterior hypothalamus

**PFH:WA(Orx).** Orexin-containing neurons are distributed in the PFH (Sakurai et al. 1998). Activation of these neurons is positively correlated with wakefulness and negatively correlated with sleep (Estabrooke et al. 2001). Many WA neurons exist in this area (Alam et al. 2002; Koyama et al. 2003). Recently, the orexin-containing neurons have been shown to be WA (Lee and Jones 2004; Mileykovskiy et al. 2005). They send excitatory projections to the neural groups in the LDT (Nambu et al. 1999; Peyron et al. 1998). In addition, they are self-excited via glutamatergic neurons (Yamanaka et al. 2002a) and inhibited by the N-R(GABA) neurons in the POA (Alam et al. 2005; Gong et al. 2002; Sakurai et al. 2005). The putative function of WA(Orx) neurons is the maintenance of wakefulness (Sakurai 2000). Although W-R and REM-on neurons also exist in the PFH, they are thought to be under the control of the brain stem (Koyama et al. 2003).

**TMN:WA(HA).** Neurons of this group are located in the TMN (Lin et al. 1996; Sakai et al. 1990; Steininger et al. 1999) and are excited by the WA(Orx) and WA(NA) neurons and inhibited by the REM-on(GABA) neurons and N-R(GABA) neurons in the POA (Nitz and Siegel 1996; Yamanaka et al. 2002b; Yang and Hatton 1997). They have projections into the cortex, thalamus, and the brain stem arousal systems, which contribute to the maintenance of wakefulness.

**POA**

**VLPO:** Neurons in this group are distributed in the VLPO (Sherin et al. 1996). They are activated by sleep-promoting substances (Chamberlin et al. 2003; Moraitry et al. 2004). They are most active during NREM sleep and comparably or less active during REM sleep (though this state-dependency is not statistically significant) (Szymusiak et al. 1998). Anatomically, these neurons have reciprocal projections from and to arousal systems such as those originating in the LC, TMN, PFH, and the raphe nuclei (McInty and Szymusiak 2003). Noradrenaline and serotonin exert inhibitory effects on the VLPO neurons (Gallopin et al. 2000). These neurons also receive cholinergic inhibition whose origin is thought to be the mesopontine tegmentum (Gallopin et al. 2000; Saint-Meux et al. 2004).

**MnPN:** Neurons in this group are distributed in the MnPN. Their activities are under the control of sleep-promoting substances (Baker et al. 2005) as well as brain temperature (Gong et al. 2000). They are activated at the beginning of NREM sleep and decrease activity until the end of NREM sleep and are activated during REM sleep (Suntsova et al. 2002). Similar to the VLPO neurons, these neurons have reciprocal projections from and to the arousal systems (McGinty and Szymusiak 2003). Physiologically, noradrenaline evokes inhibitory responses in GABAergic neurons in the MnPN (Bai and Renaud 1998).

**Other sleep-active neurons.** In the preoptic/anterior hypothalamic area, there exist neurons whose activities are selectively high during NREM sleep (Koyama and Hayashi 1994; Osaka and Hayashi 1995). However, their physiological properties, such as neurotransmitters and axonal projections, are not clearly known.

**Basal forebrain**

**BASAL FOREBRAIN:W-R(ACH).** Neurons in this group are excited by the TMN:WA(HA) and noncholinergic neurons in the brain stem (Jones 2004; Khateb et al. 1995; Ramesh et al. 2004). During REM sleep and wakefulness, they activate the hippocampus, thalamus, and cortex through ascending projections.

**BASAL FOREBRAIN:N-R(GABA).** These neurons participate in the initiation and maintenance of NREM sleep in association with the N-R(GABA) neurons in the POA (Gritt et al. 1994).

**Circadian controls**

Neurons in the suprachiasmatic nucleus (SCN), the center of circadian rhythms, increase activity during subjective day and reduce activity during subjective night in both nocturnal and diurnal animals (Inouye and Kawamura 1982; Sato and Kawamura 1984). There are indirect projections from the SCN to the sleep- and arousal-promoting systems in the brain stem (Aston-Jones et al. 2001; Chou et al. 2003; Deurveilher and Semb 2003, 2005).

**Sleep-promoting substances**

Various types of sleep-promoting substances (SPSs) have been considered to be involved in the homeostatic regulation of sleep and wakefulness. Among them, prostaglandin D2 (PGD2) affects the ventral surface of the rostral basal forebrain, and its sleep-promoting effect is mediated by adenosine (Chamberlin et al. 2003; Hayashi and Urade 2001; Moraitry et al. 2004). Delta sleep-inducing peptide (DSIP) and various cytokines selectively increase NREM sleep (Alam et al. 2004; Davenne and Krueger 1987; Tobler and Borbély 1980), whereas GSSG (oxidized glutathione) and PGD2 promote REM sleep and NREM sleep (Honda et al. 1994). Empirically, REM sleep and NREM sleep are suggested to exhibit their own homeostatic regulation (Ocampo-Garcas et al. 2000; Rechtschaffen et al. 1999). Although the mechanisms of these regulations are not known in detail, they may involve some molecular processes other than the SPSs.

**METHODS**

We used the actual neuronal activities across the state transitions recorded from the PFH and the brain stem in rats. These data were obtained in our previous experiments (Koyama et al. 2001, 2003).
Here, for the reader’s reference, the experimental procedures are summarized.

For the recordings, the experiments were performed on nine unanesthetized male rats (300–450 g, Sprague-Dawley, Japan SLC, Shizuoka, Japan), which were housed under 12-h light/dark conditions (light on at 7:00 a.m.). The rats’ heads were restrained as described elsewhere (Koyama et al. 2001). The electrodes for EEG and neck EMG activity were implanted under pentobarbital sodium anesthesia (50 mg/kg). After ≥7 days of recovery from surgery, the rats were deprived of sleep for 12 h in a slowly rotating wheel (40 cm diam, 0.7 rpm) with food and water available ad libitum. They were placed in plastic boxes sized to their bodies, and their heads were fixed to the stereotaxic frame with specially devised plates. The recording started around noon and lasted ≤7 h. Single neuronal activity was recorded extracellularly through a glass pipette microelectrode, amplified, and filtered with a cut-off frequency of 53 Hz. The activity was digitized at a sampling rate of 10 kHz with a CED 1410 data processor (Cambridge Electronic Design). After the experiment, to locate the recorded neurons, appropriate procedures were used for histology and orexin immunohistochemistry.

Sleep staging criteria

To detect the moment of state change, the sleep staging criteria used here are different from those used in either Koyama et al. (2001) or Koyama et al. (2003). The timing of state change was determined by visual inspection based on EEG and EMG activities. NREM sleep → REM sleep is determined by the dominant appearance of sustained theta waves, NREM sleep → wakefulness by reduction of EEG amplitude and increase in EMG activity, REM sleep → wakefulness primarily by increase in EMG activity, and wakefulness → NREM sleep by the appearance of delta waves in the EEG. Note that the standard epoch-wise scoring criteria are not suitable for our purposes.

Classification of state dependency of neuronal activity

The quantitative criteria used here to classify neuronal activities are given below. They are different from those in either Koyama et al. (2001) or Koyama et al. (2003). A neuron is classified in the WA group if its mean firing rate is maximal during wakefulness and is <25% of the maximum during REM sleep. The REM-on neuron’s mean firing rate is maximal during REM sleep, whereas those during the other states are <50% of the maximum. A neuron is categorized in the W-R group when the state dependency of its firing rate satisfies the following criteria: the mean firing rate during either wakefulness or REM sleep is maximal, the ratio of mean firing rates between wakefulness and REM sleep is 1.2 or closer, and the mean firing rate during NREM sleep is <50% of the maximum. According to these criteria, 6 WA neurons were classified as in the PFH and 20 REM-on and 17 W-R neurons as in the brain stem.

All procedures were carried out under the guidance of the Animal Research Experiments in accordance with the Guideline on Animal Experiments in Fukushima Medical University and Japanese Government Animal Protection and Management Law.

RESULTS

Modeling of regulatory mechanisms of sleep and wakefulness

Here, we construct a model of the mechanisms regulating sleep-wakefulness based on the recent physiological findings described above. The model is composed of formal neurons, each of which represents the collective activities of neurons in the same group. This is not a crude approximation. Under the appropriate statistical conditions, the macroscopic activity of the neural network whose component neuron integrates many inputs has been shown to behave like a formal neuron (Amari 1971). The neuron groups included are VLPO:N-R(GABA), MnPN:N-R(GABA), PFH:WA(Orx), TMN:WA(HA), basal forebrain (BF):W-R(Ach), LC:WA(NA), DR:WA(SHT), BS:REM-on(Ach), BS:REM-on(nonaCh), and BS:W-R(Ach), where the model neuron is distinguished from the actual neurons by use of italic letters. Based on the physiological findings summarized above, these neurons are coupled to construct the model. In addition, autoexcitation mechanisms are introduced in PFH:WA(Orx), LC: WA(NA), and TMN:WA(HA) neurons, because they exhibit spontaneous firing even in slice preparations of rat brain (Kamondi and Reiner 1991; Williams and Marshall 1987; Yamanaka et al. 2003). Two kinds of SPSs, SSI and SS2, are also included to implement the homeostatic regulation of sleep and wakefulness. SSI is accumulated during wakefulness, activates VLPO:N-R(GABA), and is dissipated during sleep. In contrast, SS2 is accumulated during wakefulness and REM sleep, activates MnPN:N-R(GABA), and is dissipated during NREM sleep. The rates of accumulation and dissipation of SS2 are higher than those of SSI. Figure 2A depicts the model configuration in detail. The mathematical representation of the model is given in the APPENDIX.

In modeling, some simplifications and tentative assumptions have been made, as follows. The REM-on and W-R neurons in the PFH are not distinguished from those in the brain stem, because no discharge patterns unique to them could be observed (Koyama et al. 2003). The BF:N-R(GABA) neurons are not distinguished from those in the POA. The sleep-active neurons activated exclusively during NREM sleep in the preoptic/anterior hypothalamus are not explicitly included in the model because their function can be covered by the VLPO:N-R(GABA) and MnPN:N-R(GABA) neurons (see DISCUSSION). The REM-on(Glu) and REM-on(GABA) neurons distributed in the brain stem are merged into BS:REM-on(nonaCh), as a result of which BS:REM-on(nonaCh) can exert excitatory as well as inhibitory effects on target neurons. Therefore REM-on inhibition is executed by BS:REM-on(nonaCh) in the model. This, of course, does not conform to physiological findings. However, this would not be a crude simplification if the REM-on(GABA) neuron only reverses the sign of excitatory effect of the REM-on(Glu) neuron. As noted above, the existence of REM-on(GABA) neurons is still controversial. Addressing this physiological issue is beyond the scope of this paper; however, from the modeling point of view, whatever the mechanism underlying “REM-off activity” is, appropriate “REM-on” suppression is functionally needed although it does not have to be GABAergic. Therefore such a “REM-on” inhibition is tentatively introduced as described above. Another controversy concerns the monoaminergic inhibition of BS:REM-on neurons. Because, at least in the rat’s LDT, the experimental results suggest the existence of this inhibition, the BS:REM-on neurons are designed to undergo inhibitions by LC:WA(NA) and DR:WA(SHT) in the model. However, this inhibition alone does not play a decisive role in permitting REM sleep. This implementation also coincides with the idea that the cessation of monoaminergic activity is not sufficient for induction of REM sleep (Sakai 1988). Verification of this is left to future physiological studies.
groups and the autoexcitation in each group. For abbreviations, see the text. Structure can be roughly indicated by the mutual inhibitions between the neural neurons have mutual inhibitory couplings with the REM neurons. Note that while the N-R neurons have autoexcitatory mechanisms, each can be exclusively activated by mutual inhibitions between them.

Simulation of sleep and wakefulness of rats

Figure 3 shows a fundamental sleep-wakefulness pattern generated by the model. A bidirectional state change between wakefulness and NREM sleep, and a state change from NREM sleep to wakefulness through REM sleep, are clearly observed. Actually, a state change from REM sleep to NREM sleep may be observed under the standard scoring criteria. In our experience, even in such a case, an undetectable, transient period of wakefulness often intervenes in the state change from REM sleep to NREM sleep. These simulated state-changing patterns thus faithfully reproduce those of rats. Here, the sleep-wakefulness stages of the model are tentatively determined based on the activity levels of TMN:WA(HA) and BS:REM-on(ACh) as members of the WA and REM groups, respectively: Activity of TMN:WA(HA) > 0.6 defines wakefulness, activity of BS:REM-on(ACh) > 0.6 defines REM sleep, and otherwise, NREM sleep occurs. Notably, this staging is nearly unaffected even if the other member in the respective group is selected. The parameter values are given in the APPENDIX. By comparing the mean REM sleep durations between the actual data and the model, the time constants $\tau_7$, $\tau_8$, and $\tau_{10}$ can be estimated to be 0.15s, and the others as 0.1s. Not all of the time-courses of neuronal activity are faithfully reproduced in the model. In particular, the model neurons of the WA group are only minimally activated during NREM sleep. This result does not necessarily coincide with the finding that the WA neurons still exhibit moderate activity during NREM sleep, as shown in Fig. 1. The ON/OFF nature of neuronal states in the model is caused by the model being composed of McCulloch-Pitts-type neurons. Detailed discussion of this issue will be provided later.

To assess the modeled mechanisms of regulation of state change, various pharmacological experiments are simulated. Figure 4 summarizes the results, with the numbers on the abscissa corresponding to those of the experiments explained below: 0) simulated sleep-wake cycle in the inactive (light) period, referred to as the control (this proportion of sleep and wakefulness is similar to actual findings; e.g., Clément et al. 2003); 1) carbachol, an agonist of acetylcholine, injected into the caudal part of cat peri-LC$\alpha$, increases the proportion of REM sleep (Sakai and Crochet 2003)—this is simulated by adding 0.1 to the activity of BS:REM-on(nonACh), which results in an increased proportion of REM sleep; 2) injection of noradrenaline in cat peri-LC$\alpha$ decreases the proportion of REM sleep (Sakai and Crochet 2003)—this is simulated by subtracting 0.06 from the activity of BS:REM-on(nonACh), which results in a decreased proportion of REM sleep: 3) injection of serotonin in cat LDT decreases the proportion of REM sleep (Sanford et al. 1994)—this is simulated by subtracting 0.13 from the activity of BS:REM-on(ACh), which results in a decreased proportion of REM sleep.

The above results of simulation qualitatively reproduce experimental observations, although care in interpretation is required because of differences among species. This coincidence between the results of simulation and experimental findings supports the physiological reality of the model structure. In addition to these acute experiments, the behavior of genetically manipulated mice is simulated. Genetically orexin-
ablated mice have increased proportion of REM sleep during the dark period, but their organization of sleep and wakefulness is unchanged during the light period (Hara et al. 2001; Willie et al. 2003), which is simulated by excluding \textit{PFH:WA(Orx)} from the model. The simulation shows that the proportion of REM sleep during the active (dark) period is increased without changing the organization of sleep and wakefulness during the inactive (light) period (Fig. 5).

For direct examination, the activities of the model neurons across state transitions are compared with those of actual neurons in rat PFH (Koyama et al. 2003) and brain stem (Koyama et al. 2001). Figure 6 schematically summarizes recorded neuronal discharge patterns across state transitions, from which some neuronal activities are selected for comparison with those of simulation, as shown in Fig. 7. Classification and characterization of neuronal discharge patterns has been revised based on the criteria described in the APPENDIX. The actual activities in Figs. 6 and 7 are those of the W-R and REM-ON neurons in the brain stem and a WA neuron in the PFH. The W-R neuron is thought to be cholinergic, and one type of REM-ON neuron is cholinergic and the other noncholinergic; their neurotransmitters are conjectured based on spike waveform criteria (Koyama et al. 1998). The WA neuron in the PFH is putatively an orexin neuron (Alam et al. 2002; Koyama et al. 2003; Lee and Jones 2004). Note that state change in the model does not necessarily coincide with actual state changes because of differences in the criteria for staging.

Across the transition from NREM sleep to REM sleep, the W-R and cholinergic and noncholinergic REM-ON neurons in the brain stem begin to increase their discharge rates before the state change, although there are variations in when activation starts and how fast it grows, as shown in Fig. 6. In contrast, the WA neuron is only minimally activated around the state change, but some neurons are just briefly activated. In Fig. 7A, some of the actual discharge patterns are compared with those of the model neurons, i.e., \textit{PFH:WA(Orx)}, \textit{BS:W-R(ACh)}, \textit{BS:REM-ON(ACh)}, and \textit{BS:REM-ON(nonACh)}. Naturally, the actual discharge patterns have short-term and long-term fluctua-
BS:REM-ON(Glu) neuron is slightly released from inhibition could be interpreted based on model operation, that is, the observed in some of the BS:REM-ON neurons. Differences in inhibition across the state-transition underlies the brief activation in inhibited by the LC:WA(NA) neuron. This process of disinhibition to waking, the BS:REM-ON(Glu) neuron is again prevented by the MnPN neuron as NREM sleep nearly ends. After the state change, the definition of which is given in the APPENDIX. Data for the perifornical hypothalamus (PFH:WA(Orx)) neurons are taken from Koyama et al. (2003), and those of the brain stem neurons from Koyama et al. (2001). Classification and characterization of neuronal discharge patterns are completely revised here. For abbreviations, see the text.

From wakefulness to NREM sleep, the WA neuron decreases its rate of discharge across this state transition. The W-R neuron gradually decreases its rate of discharge across the transition. In contrast, the REM-on neurons in the brain stem exhibit low activity independent of the state change. Figure 7D shows actual neuronal activity patterns across this state transition together with the results of simulation. The PFH:WA neuron gradually decreases its discharge rate well before the state change and then further decreases its rate after the state change. The BS:W-R neuron gradually decreases activity after

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the state change. The simulated neuronal activities appear roughly to follow the profiles of actual neuronal activities.

In addition to the activities of the neurons in the hypothalamus and the brain stem, those of the model neurons, VLPO: N-R (GABA) and MnPN: N-R (GABA), are examined with reference to the actual time-courses of neuronal activities in the POA over different states (McGinty and Szymusiak 2003). As shown in Fig. 8, VLPO: N-R (GABA) largely maintains its activity throughout sleep. In contrast, MnPN: N-R (GABA) decreases activity progressively during NREM sleep, and again increases activity during REM sleep. These time-courses of neuronal activity in the model coincide well with those of actual neuronal activities (McGinty and Szymusiak 2003; Suntsova et al. 2002; Szymusiak et al. 1998).

Mechanisms regulating sleep and wakefulness

The close accord in neuronal activities between the model and the actual data shows the physiological accuracy of the model. In the following, the mechanisms regulating sleep and wakefulness are explained in light of operation of the model. Figure 9 shows the organization of the neural interactions associated with changes and maintenance of state in the model.

Wakefulness

Wakefulness is maintained by the inhibition of N-R and REM neurons exerted by the WA neuron. The LC:WA(NA) neuron is further activated by mutual excitation with the W-R neuron, which is activated by the WA(Orx) and WA(HA) neurons. During wakefulness, SS1 and SS2 are accumulated, and thus the state change to NREM sleep is prepared for by activation of the N-R neuron group.

NREM sleep

During wakefulness, activation of the N-R neuron group increases, and simultaneously raises the inhibition of the WA and REM neurons exerted by the N-R neuron. When an N-R
neuron activity completely suppresses the WA and REM neurons, i.e., turns them off, the state changes from wakefulness to NREM sleep. During NREM sleep, SS1 and SS2 are dissipated at different rates: the dissipation of SS1 is slower than that of SS2. This gradually reduces N-R neuron suppression of the WA and REM neurons, which competitively determines which neuron group turns on next. That is, if reduction of suppression of the REM neuron reaches a threshold earlier than that of the WA neuron, the REM neuron turns on because of mutual excitation between REM-ON(ACh) and REM-ON(nonACh) and between REM-ON(nonACh) and W-R(ACh). Otherwise, the WA neurons turn on as a result of their autoexcitability, as supported by the excitatory interconnections among WA(NA), WA(HA), and WA(Orx) and the mutual excitation between WA(NA) and W-R(ACh).

**REM sleep**

During NREM sleep, dissipation of SS1 and SS2 inactivates the N-R neurons. After the change to REM sleep, SS1 continues to be dissipated, whereas SS2 is accumulated progressively to reactivate MnPN:N-R(GABA). Because of this, MnPN:N-R(GABA) increases suppression of the REM and WA neurons. Because the suppression by MnPN:N-R(GABA) is not sufficient to turn off the REM neurons, REM sleep is maintained by mutual excitation between REM-ON(ACh) and REM-ON(nonACh) and between REM-ON(nonACh) and W-R(ACh). REM neuron activity inhibits the WA neurons. The continuous decrease in SS1 during sleep states further weakens the suppression by VLPO:N-R(GABA) of WA neurons, releasing the WA neurons from suppression. The REM neurons are forced to turn off by suppression from the WA neurons. In this fashion, REM sleep is followed by wakefulness.

As shown above, the resulting neuronal activities and the organization of interactions are not always correlated. For example, coactivation of MnPN:N-R(GABA) and BS:REM-ON during REM sleep seems inconsistent with the expected result of unidirectional inhibition by MnPN:N-R(GABA) of BS:REM-ON. Actually, the mutual excitation between the BS:REM-ON neurons and between BS:REM-ON and W-R overcomes the inhibition by MnPN:N-R(GABA). This is a model-based interpretation of the mechanism underlying the finding that, despite activation of the N-R neurons, REM sleep persists, as shown in Fig. 1. In this fashion, the model structure discloses a hidden mechanism realizing the complex state dependency of neuronal activity patterns briefly shown in Fig. 1.

**Circadian regulation of sleep and wakefulness**

Sleep and wakefulness are regulated in circadian fashion in rats. In the model, the circadian modulators in VLPO:N-R(GABA) and MnPN:N-R(GABA) are inhibitory for the active...
Modeling of human sleep and wakefulness rhythms

Human sleep and wakefulness are characterized by their concentration in phase and rhythmic, not random, REM-NREM alternation. In addition, the human sleep-wakefulness rhythm tends to exhibit bidirectional state changes between REM sleep and NREM sleep and almost unidirectional change from wakefulness to NREM sleep and REM sleep to wakefulness. In this study, we can preserve the model structure used for rats and simulate the human sleep-wakefulness rhythm by tuning the model parameters. Concentrated sleep and wakefulness are realized by augmenting circadian modulations in the activities of VLPO:N-R(GABA), MnPN:N-R(GABA), and PFH:WA(Orx). In order for REM-NREM alternation to continue without the intervention of wakefulness, the WA neurons should be sufficiently suppressed during sleep, and MnPN:N-R(GABA) alone is necessary to force the REM neurons to turn off. To satisfy the former condition, the rates of production and dissipation of SS1 are reduced for human simulation. To meet the latter condition, inhibition by MnPN:N-R(GABA) of REM-\textit{on}(\textit{nonACh}) is enhanced. Additionally, the rate of production of SS2 during REM sleep, \( k_r \), and its rate of dissipation during NREM sleep, \( k_n \), are increased so that SS2 can change sufficiently faster than SS1, which allows several REM-NREM cycles to take place during a sleep period. The applied noise is reduced to obtain less random alternation between sleep and wakefulness. The modification of model parameters is described in the Appendix. Figure 11 shows the simulated sleep-wakefulness rhythm. The major properties of the human sleep-wakefulness rhythm are clearly reproduced:

1) sleep starts with NREM sleep;
2) sleep ends with REM sleep;
3) sleep contains several REM-NREM cycles; and
4) the duration of REM sleep is progressively prolonged. In this simulation, the time constants \( \tau_7, \tau_8, \) and \( \tau_{10} \) equal 5.2 s, and the others are 3.5 s.

The mechanisms underlying state changes are summarized, with special reference to how they differ from the rat model. Maintenance of each state is realized by the same mechanism as in the rat model. On the other hand, state changes are differently controlled, owing to the alteration of model parameters described above. Enhanced circadian regulation concentrates wakefulness and sleep along the time axis: wakefulness dominates when N-R(GABA)/WA(Orx) is inactivated/activated.

FIG. 10. Circadian variations in proportions of sleep and wakefulness. A: actual data (Clément et al. 2003). B: simulation. White and black bars indicate proportions in the inactive (light) and active (dark) periods, respectively. Faithful reproduction of circadian variation verifies circadian regulation implemented in the model.

FIG. 11. Simulation of human sleep-wake cycle by tuning of parameters of the rat model and introduction of a sinusoidal circadian input. Although neuronal activities and levels of SS1 and SS2 across sleep-wake cycles are not known, at least the sleep-waking pattern itself mimics the human one well. Staging criteria for sleep and wakefulness are the same as for the rat. Parameter set used is given in the Appendix.
and sleep dominates in the opposite case. The decreased rates of production/dissipation of SS1 facilitate this concentration.

After the change to NREM sleep caused by exclusive activation of the N-R neurons, VLPO:N-R(GABA) suppresses LC: WA(NA) until SS1 is completely dissipated. In contrast, SS2 is dissipated quickly during NREM sleep, which changes the state to REM sleep by reducing the inhibition of BS:REM-on(nonACh) exerted by MnPN:N-R(GABA). This one-way state change from NREM sleep to REM sleep results from the organization of the human model, in which MnPN:N-R(GABA) can by itself completely suppress BS:REM-on(nonACh). During REM sleep, SS2 is accumulated quickly. The accumulated SS2 changes the state to NREM sleep again by activating MnPN:N-R(GABA). In this way, alternations between NREM sleep and REM sleep persist until the end of sleep. This cycle can end by state change from either NREM sleep or REM sleep to wakefulness. Nevertheless, because the suppression of WA neurons during REM sleep is stronger than during NREM sleep because of cooperative inhibition by VLPO:N-R(GABA) and MnPN:N-R(GABA), the activity of WA neurons could be suppressed by the REM neurons longer than the N-R neurons if the activity of VLPO:N-R(GABA) decreases at a constant rate. Accordingly, REM sleep tends to end the cycle.

**DISCUSSION**

We constructed a model of the mechanisms regulating sleep and wakefulness. This model consists of a quartet of neuron groups including WA, N-R, REM, and W-R, which are represented by the formal neurons. The neurons of the WA and REM groups have their own direct/indirect self-excited feedback loops and mutual excitatory connections with the W-R neurons. The N-R neurons are activated by the SPSS underlying the homeostatic regulation of REM sleep, NREM sleep, and wakefulness. Mutual inhibition exists between the N-R and WA neurons and between the REM and WA neurons, and there is unidirectional inhibition of the REM neurons exerted by the N-R neurons. This model closely reproduces the sleep-waking patterns of the rat, including the bidirectional state change between wakefulness and NREM sleep and the unidirectional state changes from NREM sleep to REM sleep and from REM sleep to wakefulness. The physiological accuracy of the model was verified by simulating the results of pharmacological experiments and sleep-waking patterns with genetic ablation of orexin neurons. More directly, the activities of the model neurons were compared with those of the actual neurons across state transitions. The rough agreement between the model and actual neuronal activities show that the dynamics of the model resemble those of the actual neural systems controlling the sleep-wake rhythm. In addition, strengthening the mutual inhibition between the N-R and WA neurons and reducing noise enabled the model to simulate human sleep-wake rhythms without changing the fundamental model structure. These results show that the model exhibits the primary properties of the mechanisms regulating sleep and wakefulness.

One of the predictions of the model is the existence of differences in function between the MnPN:N-R and VLPO:N-R neurons, which has not yet been clarified physiologically. As previously noted, the physiological and anatomical properties of the MnPN:N-R and VLPO:N-R neurons are similar, with the exception of state-dependency of neuronal activity pattern, response to cholinergic input, and thermosensitivity related to sleep control (Gallopin et al. 2000; McGinty and Szymusiak 2003). In the model, MnPN:N-R(GABA) and VLPO:N-R(GABA) play primary roles in changing NREM sleep to REM sleep and wakefulness, respectively, in addition to their function of inducing sleep. Their functions are differentiated by the following model structure. VLPO:N-R is activated by SS1, and MnPN:N-R by SS2. Both neurons inhibit the arousal system. In addition, MnPN:N-R exclusively inhibits BS:REM-on(nonACh). This also explains how the state-dependent activities of the VLPO:N-R and MnPN:N-R neurons are generated. Another type of N-R neuron exists, which is activated exclusively during NREM sleep (Koyama and Hayashi 1994). This type of sleep active neuron is expected to play a role in inducing/maintaining NREM sleep or in generating NREM-related physiological events. Nevertheless, at least within the framework of our model, existence of this type of neuron is not thought to force us to reconstruct the implemented mechanism regulating sleep and wakefulness, because NREM-specific activation could be produced by appropriate combinations of model components, e.g., VLPO:N-R/MnPN:N-R and BS:REM-on suppression. This is why this type of neuron was not explicitly included in the model.

The REM–NREM cycle was first modeled as a prey–predator system of the REM–ON and WA neurons (Massaquoi and McCarley 1992; McCarley and Hobson 1975; McCarley and Massaquoi 1986b). Recently, new findings for sleep active neurons and wake-related neurons have led researchers to interpret the sleep-waking mechanism as a bistable system, i.e., a flip-flop system (McGinty and Szymusiak 2000; Saper et al. 2001). Nevertheless, how the flip-flop is flipped has not been clearly elucidated. In addition, no integrated neural network model of REM-NREM-wakefulness regulation has yet been proposed. Recent anatomical and physiological findings suggest the inclusion of bistable subsystems in the neural networks involved in sleep-wake regulation. A simple bistable system is realized by mutual inhibition between neurons that have autoexcitation mechanisms such as positive feedback loops. When each “neuron” is replaced by a network consisting of neuronal populations, this basic bistable system could be almost the same (Amari 1971). As is clear, such bistable systems are multiply embedded in the networks shown in Fig. 2, which readily suggests the flip-flop interpretation. Nevertheless, a simple flip-flop realizes only a bidirectional change, which does not necessarily agree with actual state change behavior. The system regulating sleep and wakefulness can thus be regarded as a multistable system whose equilibrium states are not symmetrically commutative. Our model provides one possible quantitative implementation of such a peculiar multistable system, which is composed of interconnected multiple flip-flops and the SPSS that play various roles in homeostatic regulation. In fact, not all of the mechanisms of such a system, including the synthesis and reception of the SPSS, are fully understood. Further findings will be needed to verify the mechanisms implemented in the model. There is also the possibility that other chemical agents such as NO and their associated intracellular signaling pathways play the roles of SS1 and/or SS2 (Chen et al. 2003; Clément et al. 2004; Datta et al. 1997; Monti and Jantos 2004). 

In our framework of modeling, VLPO:N-R(GABA) and MnPN:N-R(GABA) flip the multistable system of sleep and
wakefulness with the aid of the SPSs. To show the behavior of the multistable system of the rat, a tridimensional trajectory constructed from the activities of VLPO:N-R(GABA), MnPN:N-R(GABA), and REM-on(nonACh)/WA(NA) is traced for the state sequences of wakefulness → NREM sleep → REM sleep → wakefulness → . . . wakefulness → NREM sleep → wakefulness → . . . as shown in Fig. 12. These trajectories are obtained under noise-free conditions. The state sequence of wakefulness → NREM sleep → wakefulness → . . . is consistently generated by changing $W_{95}$ to $-6$ and $k_{62}$ to 0.22; for the sequence of wakefulness → NREM sleep → REM sleep → wakefulness → . . . , $W_{95}$ is only changed to $-6$. The trajectories for wakefulness are $a \rightarrow b \rightarrow c$ and $e \rightarrow i \rightarrow f$, for NREM sleep, they are $c \rightarrow d \rightarrow e$ and $i \rightarrow j \rightarrow g$, and for REM sleep, they are $e \rightarrow f \rightarrow a$. In Fig. 12B, how the activities of VLPO:N-R(GABA) and MnPN:N-R(GABA) determine the next state after NREM sleep is shown. The activity of VLPO:N-R(GABA) just before the state change is higher with change to REM sleep than with change to wakefulness, as is shown by the trajectories $c \rightarrow d$ and $i \rightarrow j$ during NREM sleep.

Our model may still be too simple physiologically. One of our principal simplifications is use of a formal neuron model. Each formal neuron in the network tends to take either of two neuronal states. This is why the moderate activity of WA neurons during NREM sleep is not faithfully reproduced in the model. One way of dealing with this problem may be representation of the dynamics of neural populations by the population dynamics of more physiology-based neuron elements, such as a spiking neuron model (Gerstner and Kistler 2002). This will be a future subject of our research. Nevertheless, this study provides a possible quantitative mechanism for the flipping of this multistable system, which could function as a novel framework for understanding narcolepsy and other sleep disorders.

**APPENDIX**

**Model of rat sleep-wakefulness regulation**

The model consists of 10 formal neurons ($X_1, \ldots, X_{10}$) and 2 sleep-promoting substances (SS1 and SS2). The mathematical expression and the parameter values are given as follows

$$X_i = f(u_i, \{i = 1, \ldots , 10\}) \quad (A1)$$

$$\frac{du_i}{dt} = \frac{W_{ij}X_j}{\tau_i} - \theta_i + \delta_1(SS1 + C_1) + \delta_2(SS2 + C_2) + \delta_3 \cdot C_j + \xi_i \quad (A2)$$

$$f(u) = \frac{1}{1 + \exp\left(\frac{u - \mu_i}{\tau_i}\right)} \quad (A3)$$

where the notations $X_1, \ldots, X_{10}$ represent the activities of VLPO:N-R(GABA), MnPN:N-R(GABA), PFF:WA(Orx), TMN:WA(NA), LC:WA(NA), DR:WA(SHT), BS:W-R(ACh), BS:REM-on(ACh), BS:REM-on(nonACh), and BS:W-R(ACh), respectively. In addition, SS1 and SS2 denote the SPSs modulating the activities of VLPO:N-R(GABA) and MnPN:N-R(GABA), respectively. $W_{ij}$, $\tau_i$, $\theta_i$, and $\mu_i$ are synaptic weights from the $j$th neuron to the $i$th, time constants, thresholds, and slope factors of the activation function $f(u)$, respectively. $\delta_1$ is Knoecker’s delta, i.e., it takes the value 1 only if $i = j$, and otherwise is equal to 0. $\xi_i$ is a zero-mean Gaussian noise whose SD is 0.5. $C_1$ ($i = 1, 2, 3$) is a circadian modulator; for the active period, $C_1$, $C_2$, and $C_3$ are $-2.2$, $-2.8$, and 0.05, respectively, whereas for the rest period, they are null. The other parameter values are given as follows

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**FIG. 12.** Model trajectories constructed for VLPO:N-R(GABA), MnPN:N-R(GABA), and REM-on(nonACh)/LC:WA(NA). A: 3-dimensional trajectories constructed for the state sequence, wakefulness → NREM → wakefulness (gray), is constructed with VLPO:N-R(GABA), MnPN:N-R(GABA), and LC:WA(NA), and that for the sequence wakefulness → NREM → REM → wakefulness (black) is constructed with VLPO:N-R(GABA), MnPN:N-R(GABA), and REM-on(nonACh). B: projections of the trajectory on the VLPO:N-R(GABA)–MnPN:N-R(GABA) plane. C: projections on the MnPN:N-R(GABA)–REM-on(nonACh)/LC:WA(NA) plane. D: projections on the VLPO:N-R(GABA)–REM-on(nonACh)/LC:WA(NA) plane. To enhance 3-dimensional view, letters show corresponding points in each projection, where ◦ indicates an almost perpendicular trajectory from the front to the back and ⊙ from the back to the front. For example, $a \perp b$ means that the trajectory passes $a$ and approaches perpendicularly to $b$.  

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The dynamics of SS1 and SS2 are described as follows. SS1 is accumulated during wakefulness and otherwise dissipated. SS2 is accumulated during wakefulness and the REM state, and otherwise dissipated:

$$
\frac{dSS1}{dt} = \delta_{wake}w_1 - \delta_{sleep}k_1 \times SS1 - k_1 \times SS1 + \eta_1 \quad (A4)
$$

$$
\frac{dSS2}{dt} = \delta_{wake}w_2 + \delta_{REM}k_2h_1 - \delta_{REM}k_2h_2 - k_2 \times SS2 - k_2 \times SS2 + \eta_2 \quad (A5)
$$

where $\delta_{wake}$ takes the value 1 if the network is in the specified state, and otherwise is 0. $k_1$, $k_2$, $k_3$, $k_4$, $k_{11}$, and $k_{12}$ are the constants 0.15, 0.15, 0.015, 0.06, 0.01, 0.03, and 0.03, respectively. $h_1$ and $h_2$ are zero-mean Gaussian noises whose SD is 1.0.

The equations are solved numerically by the fourth-order Runge-Kutta method with a time resolution of 0.02 common to rats and humans.

Model of human sleep-wakefulness regulation

To model human sleep-wakefulness regulation, some of the model parameters are modified as follows: $W_{57}: 0.9 \rightarrow 0.8$, $W_{66}: -3.0 \rightarrow -4.0$, $W_{86}: 2.0 \rightarrow 0.5$, $W_{92}: -2.0 \rightarrow -4.0$, and $W_{95}: -4.5 \rightarrow -5.0$.

The circadian modulators are replaced by 2.2 $\left(\sin \frac{2\pi}{24} + 1\right)$ for $C_{1t}$, 2.8 $\left(\sin \frac{2\pi}{24} + 1\right)$ for $C_{2t}$, and 0.05 $\left(\sin \frac{2\pi}{24} + 1\right)$ for $C_{3t}$. In addition, the SD is changed from 1.0 to 0.1.$k_{w1}$, $k_{w2}$, $k_{w3}$, $k_{w4}$, and 0.001, respectively.

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