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Ein mathematisches Modell von Schlaf-Wach-Zyklen: die Rolle von Hypocretin/Orexin für homöostatische Regulation und Thalamische Synchronisation

A mathematical model of sleep-wake cycles: the role of hypocretin/orexin in homeostatic regulation and thalamic synchronization

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Zusammenfassung

Schlaf ist von vitaler Bedeutung für unsere Gesundheit und unser Wohlbefinden. Trotzdem sind fundamentale Fragen hierzu nach wie vor ungeklärt, z.B. "warum schlafen wir?" oder "welches sind die Mechanismen der Schlaf-Regulation?". Ein besseres Verständnis der Schlafregulation könnte neue Perspektiven eröffnen, sowohl für die neurophysiologische Grundlagenforschung als auch für die Behandlung von Schlafstörungen.

Ein allgemein anerkanntes Konzept der Schlafregulation wurde 1982 von Alexander Borbély vorgeschlagen. Danach ergeben sich Schlaf-Wach-Zyklen aus der Wechselwirkung zwischen einem zirkadianen und einem homöostatischen Prozess. Der zirkadiane Prozess wird einer "genetischen Uhr" in den Neuronen im Nucl. Sprachiasmaticus des Hypothalamus zugeschrieben, während die Mechanismen des homöostatischen Prozesses noch ungeklärt sind.

Mit dieser Arbeit wird ein neues Konzept einer durch Hypocretin (Orexin) vermittelten Schlaf-Homöostase vorgestellt. Das Neuropeptid Hypocretin ist ein synaptischer Ko-Transmitter von Neuronen im lateralen Hypothalamus und ist von besonderer Bedeutung für die Aufrechterhaltung des Wachzustands. Unterfunktion des Hypocretin Systems führt zur Schlafkrankheit Narkolepsie, welche durch Störungen der Schlaf-Wach-Zyklen mit plötzlichen Schlafattacken am Tag und Unterbrechungen des Nacht-Schlafs gekennzeichnet ist. Anderseits wird durch Hypocretin Injektion die Wachheit und die Leistungsfähigkeit nach Schlafentzug erhöht.

Die wichtigsten Annahmen der hier vorgelegten Studie sind folgende: 1) Das Neuropeptid Hypocretin ein entscheidender Faktor der homöostatischen Schlafregulation. 2) Die kontinuierliche Impuls-Aktivität der Hypocretin Neurone im Wachzustand wird durch reziproke erregende Verbindungen mit anderen Neuronen, einschließlich lokaler glutamatergen Interneuronen, aufrechterhalten; 3) Im Verlauf anhaltender Impulsaktivität wird die synaptische Hypocretin-Wirkung vermindert bis die Aktivität im erregenden Rückkopplungskreis unterbrochen wird. 4) In der dadurch eingeleiteten, weitgehend aktivitätsfreien (Schlaf-) Phase kann sich die synaptische Effizienz wieder aufbauen, wodurch die Wiedereinstellung kontinuierlicher Aktivität (Wachzustand) durch einen erregenden Reiz, z.B. aus den circadianen Schrittmacherneuronen, zunehmend erleichtert wird.

Dieses Konzept wurde in ein mathematisches Schlaf-Modell übertragen, das

auf einem physiologisch fundierten, wenn auch vereinfachten Hodgkin-Huxley Ansatz aufbaut. Ein Hypocretin Neuron ist mit einem lokalen Interneuron über glutamaterge Synapsen in einer exzitatotorischen reziproken Rückkopplungsschleife verbunden. Es innerviert gleichfalls zwei gap junction gekoppelte thalamische Neurone und nutzt als zusätzlichen Ko-Transmitter das Neuropeptid Hypocretin. Dessen synaptische Effektivitt verringert sich mit jedem Aktionspotential und der damit verbundenen Transmitter-Ausschüttung. Diese Effekte werden von einem langsamen Erholungsprozess überlagert. Die im aktiven Wachzustand abnehmende synaptische Stärke führt zu einem zunehmenden Schlafdruck, der durch den Aufbauprozess in der aktivitätsfreien Schlafphase wieder verringert wird.

Die Computer-Simulationen können die wesentlichen Komponenten homöostatischen Veränderungen während der Schlaf- und Wachphase nachbilden, einschließlich der Wirkung äußerer Weckreize, eines Zwischenschlafs oder Schlafentzugs. Unter dem Einfluss zirkadianer Effekte kann das Computer-Modell auch die typischen Veränderungen der Impulsaktivität in hypothalamischen und thalamischen Neuronen im Verlauf von Schlaf-Wach-Zyklen wieder geben. Diese Simulationsergebnisse stützen das hier vorgelegte Konzept, wonach synaptische Veränderungen im Hypocretin System eine wichtige Komponente homöostatischer Schlaf-Wach-Regulation darstellen, auch wenn nicht auszuschließen ist, dass zusätzliche Mechanismen berücksichtigt werden müssen.

Summary

Sleep is vital to our health and well-being. Yet, we do not have answers to such fundamental questions as "why do we sleep?" and "what are the mechanisms of sleep regulation?". Better understanding of these issues can open new perspectives not only in basic neurophysiology but also in different pathological conditions that are going along with sleep disorders and/or disturbances of sleep, e.g. in mental or neurological diseases.

A generally accepted concept that explains regulation of sleep was proposed in 1982 by Alexander Borbély. It postulates that sleep-wake transitions result from the interaction between a circadian and a homeostatic sleep processes. The circadian process is ascribed to a "genetic clock" in the neurons of the suprachiasmatic nucleus of the hypothalamus. The mechanisms of the homeostatic process are still unclear.

In this study a novel concept of hypocretin (orexin) - based control of sleep homeostasis is presented. The neuropeptide hypocretin is a synaptic co-transmitter of neurons in the lateral hypothalamus. It was discovered in 1998 independently by two different groups, therefore, obtaining two names, hypocretin and orexin. This neuropeptide is required to maintain wakefulness. Dysfunction in the hypocretin system leads to the sleep disorder narcolepsy, which, among other symptoms, is characterized by severe disturbances of sleep-wake cycles with sudden sleep-attacks in the wake period and interruptions of the sleep phase. On the other hand injection of hypocretin promotes wakefulness and improves the performance of sleep deprived subjects.

The major proposals of the present study are the following: 1) the homeostatic regulation of sleep depends on the dynamics of a neuropeptide hypocretin; 2) ongoing impulse generation of the hypocretin neurons during wakefulness is sustained by reciprocal excitatory connections with other neurons, including local glutamate interneurons; 3) the transition to a silent state (sleep) is going along with an activity-dependent weakening of the hypocretin synaptic efficacy; 4) during the silent state (sleep) synaptic efficacy recovers and firing (wakefulness) can be reinstalled due to the circadian or other input.

This concept is realized in a mathematical model of sleep-wake cycles which is built up on a physiology-based, although simplified Hodgkin-Huxley-type approach. In the proposed model a hypocretin neuron is reciprocally connected

with a local interneuron via excitatory glutamate synapses. The hypocretin neuron additionally releases the neuropeptide hypocretin as co-transmitter. Besides of the local glutamate interneurons hypocretin neuron excites two gap junction coupled thalamic neurons. The functionally relevant changes are introduced via activity-dependent alterations of the synaptic efficacy of hypocretin. It is decreasing with each action potential generated by the hypocretin neuron. This effect is superimposed by a slow, continuous recovery process. The decreasing synaptic efficacy during the active wake state introduces an increasing sleep pressure. Its dissipation during the silent sleep state results from the synaptic recovery.

The model data demonstrate that the proposed mechanisms can account for typical alterations of homeostatic changes in sleep and wake states, including the effects of an alarm clock, napping and sleep deprivation. In combination with a circadian input, the model mimics the experimentally demonstrated transitions between different activity states of hypothalamic and thalamic neurons. In agreement with sleep-wake cycles, the activity of hypothalamic neurons changes from silence to firing, and the activity of thalamic neurons changes from synchronized bursting to unsynchronized single-spike discharges. These simulation results support the proposed concept of state-dependent alterations of hypocretin effects as an important homeostatic process in sleep-wake regulation, although additional mechanisms may be involved.

Chapter 1

INTRODUCTION

Sleep is a one of the most universal phenomena in biology. It is vital to our health and well-being. Yet, we do not have answers to such fundamental questions as "why do we sleep?" and "what are the mechanisms of sleep regulation?". The present study addresses the questions of sleep regulation by the brain. In particular, it is related to the mechanisms underlying the transitions between the states of sleep and wakefulness and their homeostatic control.

Historically, it was assumed that during sleep the brain is inactive and the central nervous system is shut down. Nowadays, we know that this is not the case. The brain is active during both wakefulness and sleep, although in quite different ways. This was first observed in the changes of cortical activity as a function of sleep and waking (Caton, 1875). These groundbreaking experiments were done by the English scientist Richard Caton who has made recordings directly from the cortical surface of the rabbit and monkey brains.

Later, invention of electroencephalography (EEG) made it possible to make noninvasive recording from the scalp which showed that similar changes of electrical activity are appearing also in the human brain. The invention of the EEG was a first major breakthrough in the field of sleep research. The introduction and first application of EEG technology is attributed to the German neurologist and psychiatrist Hans Berger (Berger, 1929). In the papers published between 1929 and 1938 he had demonstrated EEG responses in humans evoked by different sensory stimuli and had described the EEG rhythms that are seen during sleep and wakefulness.

It was further recognized that electrical activity of the brain and other phys-

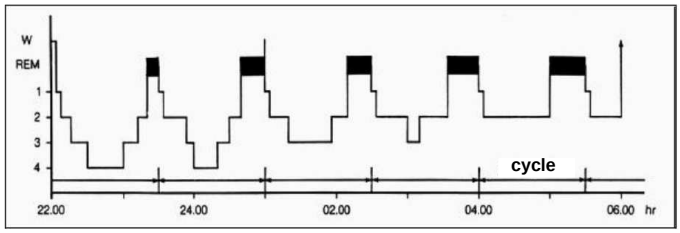

Figure 1.1: A characteristic hypnogram derived from an all-night sleep recording of healthy person. It shows the descent from waking (W) through the stages 1,2,3 and 4 of NREM sleep within the first hour of sleep, dwelling at the stage 4 for some time and ascending to the REM sleep. The cycle is repeating approximately every 90 minutes showing less deep sleep and longer REM sleep episodes as the night progresses.

iological markers also change in the course of sleep (Aserinsky and Kleitman, 1953). Accordingly, sleep was subdivided into rapid eye movement (REM) and non-rapid eye movement (NREM) sleep (Dement and Kleitman, 1957). These stages are characterized by signals of the EEG, EMG (electmyogram) and EOG (electrooculogram). During the REM sleep EEG recordings are similar to those during wakefulness, demonstrating low amplitude and high frequency waves. The REM sleep can be clearly distinguished by rapid eye movements and muscle atonia (with occasional muscular twitches). During NREM sleep the EEG recordings show synchronized waves of high amplitudes and low frequencies. Due to these low frequencies in EEG the NREM sleep is also called slow wave sleep (SWS).

Transitions between REM and NREM stages reflect the cyclic structure of sleep. Each cycle lasts for approximately 90 minutes, which means 3 to 6 cycles during a typical human sleep time of 5 to 9 hours. According to the scoring system of Rechtschaffen and Kales (Rechtschaffen and Kales, 1968), the NREM sleep is further subdivided into four stages (stage 1-4), which are differentiated by the amplitude and frequency of the EEG waves, and appearance of spindles. The characteristic hypnogram of such transitions is shown in Figure 1.1.

Sleep stage 1 is also called light sleep and is mostly appearing in a very beginning of sleep and right before awakening. Stage 2 follows stage 1 after transition to sleep. The time spent in stage 2 is increasing as the night progresses, being longest in the second half of sleep. Stages 3 and 4 are also called deep sleep. As it is seen from the hypnogram, these stages are dominating in the first hours of

sleep but disappearing towards awakening.

1.1 Sleep-related brain areas

The alterations of the EEG signals pose the questions, "how the EEG waves are generated?", and "what brain structures are involved?". The first hints came in the beginning of twentieth century from anatomical studies of the brains of patients who were suffering from encephalitis letargica. The long-term consequences of this disorder included diverse disturbances of sleep regulation such as insomnia, narcolepsy, etc.

In 1930 Constantin von Economo had published his famous work where he had related these specific disorders to damages of specific brain areas (von Economo, 1930). He has shown that lesion at the junction between the brain stem and the forebrain results in the extensive sleepiness, while lesion in the anterior hypothalamus results in insomnia. Also he has suggested that narcolepsy is caused by lesion in the lateral hypothalamus. Based on his studies, von Economo proposed the existence of the ascending arousal system, which originates in the brain stem and keeps the forebrain awake.

The idea of the ascending arousal system was later confirmed by other studies. In 1949 the two scientists Giuseppe Moruzzi and Horace Magoun demonstrated that stimulation of a specific area in the brain stem of a sleeping animal induces EEG waves that correspond to wakefulness (Moruzzi and Magoun, 1949). The nuclei of a brain stem that are involved in regulation of sleep and wakefulness are part of the so-called reticular formation. Therefore, the ascending arousal system is also called the ascending reticular activating system (ARAS).

The more studies have been done the more clear it became that the physiology of sleep is extremely complicated and involves cooperative action of many brain nuclei and neurotransmitters systems. Particular mechanisms of regulation of sleep and specific roles of the diverse nuclei are still not entirely understood. Figure 1.2A summarizes in a rather simplified way the major sleep-related brain regions and their connections. Figure 1.2B illustrates the typical EEG waves during wakefulness, NREM and REM sleep together with characteristic firing activity in different nuclei. The activity patterns of these nuclei and their alterations according to different behavioral states (wakefulness, NREM or REM sleep) give

an indication of their function.

Many of the thalamic and cortical neurons are active during wakefulness and all sleep states, but change their firing pattern from single spike discharges to impulse groups. These changes are going along with transitions from asynchronous to synchronized activity. Synchronized burst discharges are characteristic for NREM sleep and are associated with the appearance of slow waves of high amplitudes in EEG. The high-frequency low-amplitude EEG waves during wakefulness and REM sleep are related to the asynchronous single spike generation by thalamic and cortical neurons. The thalamus and cortex have reciprocal connections with each other and receive inputs from subthalamic regions, especially from the monoaminergic and cholinergic nuclei of the ARAS and from the hypothalamic nuclei.

In the hypothalamus, according to the activity states, there is a wake-promoting nucleus in the lateral hypothalamus (LHA), and a sleep-promoting nucleus in the ventrolateral preoptic area (VLPO). The suprachiasmatic nucleus (SCN) provides an intrinsic circadian signal. It exhibits a gradually changing firing activity with a maximum frequency towards the middle of the wake state. The SCN as well as the LHA have preferably excitatory projections to almost all brain areas. The VLPO has diverse inhibitory projections, especially to LHA and monoaminergic nuclei.

The monoaminergic neurons are mostly active during wakefulness and are involved in arousal mechanisms via their projections to the thalamus and the hypothalamus. They partly activate of wake-promoting and inhibit sleep-promoting nuclei. The reciprocal connections with the REM-promoting cholinergic neurons are particularly important for REM-NREM transitions.

A more detailed description of the specific dynamics of the diverse sleep-related brain regions is given below. More details can be found in books and reviews on sleep physiology. For example, see (Jouvet and Moruzzi, 1972; Siegel, 1990; Steriade, 1992; Lydic and Baghdoyan, 1998; Siegel, 2002; Sinton and McCarley, 2004; Luppi, 2004; Harris, 2005; Steriade and McCarley, 2005; McCarley, 2007; Monti et al., 2008).

1.1.1 Cortex

Cortical activity is not homogenous and different cortical areas may demonstrate different synchronization and firing properties. Nevertheless, there are typical al-

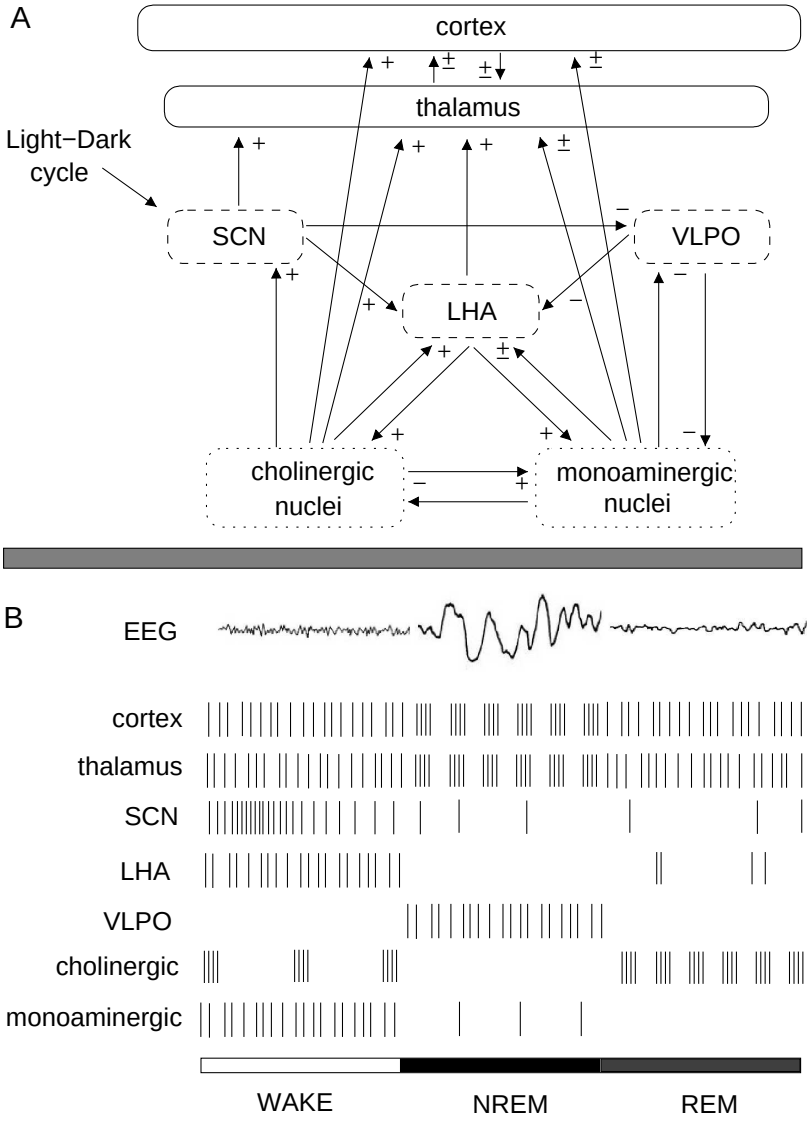

Figure 1.2: A: A simplified scheme of connections between sleep-related brain structures. B: Characteristic EEG and firing activity of these brain areas during wakefulness, NREM and REM sleep. The abbreviations are: *LHA* is a lateral hypothalamic area; *SCN* - suprachiasmatic nucleus; *VLPO* - ventrolateral preoptic nucleus; The cholinergic nuclei combine the basal forebrain and laterodorsal and pedunculopontine tegmental nuclei of brain stem; The monoaminergic nuclei include locus coeruleus, tuberomammilary nucleus,dorsal raphe nucleus and ventrolateral tegmental area. The signs of pluses and minuses refer to excitatory and inhibitory action, respectively.

terations of the firing pattern which result in the characteristic changes of EEG in different states of sleep and wakefulness (Calvet et al., 1964a; Calvet et al., 1964b; Jouvet, 1967). This characteristic changes of firing activity and corresponding EEG recordings are shown on the Fig.1.2B.

During wakefulness most of the cortical neurons are depolarized and generate action potentials (spikes) with irregular intervals (Noda and Adey, 1970). In this state the neurons fire asynchronously (Destexhe et al., 1999), what results in a typical wake EEG with low-amplitude high-frequency waves (Coenen, 1995).

During slow wave sleep the depolarization of the cortical neurons by diverse arousal centers is missing, and they start to fire groups of spikes (bursts) that are separated by longer intervals of silence (Noda and Adey, 1970). In this state the neurons seem to easier synchronize (Destexhe et al., 1999) what leads to the appearance of the EEG waves with low frequency and high amplitude. This type of EEG waves is typical for deep sleep (Coenen, 1995). During REM sleep the cortical neurons are again desynchronized. They switch to a state similar to wake activity with irregular generation of single spikes and corresponding EEG waves of high frequency and low amplitude (Destexhe et al., 1999). The cortical activity during wake and sleep states is described in many details in diverse reviews (Jouvet, 1967; Paisley and Summerlee, 1984; Dijk, 1995; McCormick and Bal, 1997; Steriade and Amzica, 1998; Steriade, 2003).

Cortical activity is crucial for determining the states of sleep and wakefulness, but their firing pattern and synchronization states essentially depend on the interactions with thalamus.

1.1.2 Thalamus

The thalamus is a center for sensory-motor integration and a major information "gate" to the cortex (Kandel et al., 1991; Blumenfeld and McCormick, 2000; Bal et al., 2000). During NREM sleep it maintains cortical synchronization and disconnects the cortex from other influences.

Thalamic neurons, similar to cortical ones, express profound alterations in activity with changes of behavioral states. In the wake state thalamic neurons fire tonically and are not synchronized with each other, while along with the transition to NREM sleep state they are switching to synchronized burst discharges (Glenn and Steriade, 1982; McCormick and Feeser, 1990).

The precise mechanism of the sleep-wake related tonic-to-bursting transitions in thalamic and cortical neurons is still unclear, although it is broadly assumed that they are driven by some extrathalamocortical processes (Steriade, 1994; Jones, 2003). The thalamic neurons receive projections from other sleep-related brain areas, among which are the hypothalamus and multiple monoaminergic nuclei of the arousal system. It is likely that interactions between extrathalamic and intrathalamic mechanisms, including intrinsic properties of thalamic neurons, contribute to the alterations of thalamic activity at the transitions from wakefulness to sleep (for reviews see McCormick and Bal, 1997; Steriade and Amzica, 1998; Steriade, 2003; Steriade and McCarley, 2005).

1.1.3 Hypothalamus

In the hypothalamus there are at least three nuclei which are important for the regulation of sleep and wakefulness. These are the suprachiasmatic nucleus (SCN), the ventrolateral preoptic nucleus (VLPO) and the lateral hypothalamic area (LHA). These nuclei have different and partly opposite roles in the control of sleep and wakefulness. (Hauta, 1946; Salin-Pascual et al., 2001; Saper et al., 2001; McGinty and Szymusiak, 2003; Saper et al., 2005; Szymusiak and McGinty, 2008).

Suprachiasmatic nucleus (SCN)

The suprachiasmatic nucleus of the hypothalamus is the master clock in the brain. It provides an endogenous circadian rhythm for diverse physiological functions, including the circadian appearance of sleep-wake cycles (for review see Meijer and Rietveld, 1989; Klein et al., 1991; Refinetti, 2005; Mistlberger, 2005; Moore, 2007). Lesion of the SCN results in random transitions between sleep and wake states, which no longer follow the day-night period of 24 hours (Ibuka and Kawamura, 1975; Edgar et al., 1993).

The circadian rhythms of the SCN neurons are manifested in their electrical activity. These neurons are most active during the subjective day and fire with lower frequencies during night. The firing rate of the SCN neurons is gradually changing in a nearly sinusoidal form (Yamazaki et al., 1998). Such circadian variation in the firing frequency is also observed in the isolated SCN neurons, but their individual periods may vary (Schaap et al., 2003; Kononenko et al., 2008).

The circadian alterations of the SCN firing rates are resulting from the activity of genetic clocks, which are present in each cell. These genetic mechanisms are widely studied. At least 12 genes, involved in circadian oscillations, have been identified. They work through promotion and/or inhibition of protein transcription and form a complex system of feedback loops which has the properties of an oscillator (Herzog et al., 1998; Albrecht, 2002).

Activity of the SCN is synchronized to the external light-dark cycle via the retinohypothalamic tract which transmits information from the light-sensitive cells in retina (Moore, 1973). This holds true even for the blind subjects (Berson et al., 2002; Provencio et al., 2002). When the retinohypothalamic tract is destroyed the circadian rhythms are still present but their periodicity might be different from 24 hours and independent from day-night cycle. Similar effects can be achieved by keeping subjects in constant darkness.

The circadian activity of the SCN together with light-dark cycles regulate synthesis of melatonin, the "hormone of darkness", in the pineal gland. In turn this hormone mediates activity of the SCN neurons and may even shift the circadian phase. Production of melatonin follows the circadian rhythm: it is lowest during day (light) and highest during night (dark). Synthesis of melatonin starts shortly before sleep and its blood level peaks about 4 hours before awakening. Synthesis of melatonin during the night can be inhibited by light, while darkness during the day cannot increase melatonin production because it is suppressed by the activity of the SCN. For detailed description see (Cassone, 1991; Illnerova, 1991; Cassone et al., 1993).

Neurons in the SCN have projections to various areas of the hypothalamus, including LHA and VLPO, as well as basal forebrain and thalamus. Major inputs to the SCN are coming from the cholinergic laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) and serotonergic dorsal raphe (DR) (for the full list of connections see Card, 1999).

Ventrolateral preoptic nucleus (VLPO)

The primary function of the VLPO in regulation of sleep and wakefulness is the promotion of sleep. Clinical and experimental studies have shown that lesions of the VLPO result in insomnia, while its stimulation enhances sleepiness (Lu et al., 2000; Saper et al., 2001). Activation of the VLPO neurons is often associated with

the action of adenosine and/or prodtoglandine D2 (Scammell et al., 1998; Urade and Hayaishi, 1999; Morairty et al., 2004; Chamberlin et al., 2003). When these substances are injected near the VLPO they induce sleepiness, while administration of adenosine receptor antagonists, such as caffeine, has a well known arousal effect (Walsh et al., 1990; Roehrs and Roth, 2008).

In contrast to the other sleep-related nuclei, neurons in the VLPO are most active during NREM and REM sleep and inactive during wakefulness (Sherin et al., 1996; Szymusiak et al., 1998). These neurons are primarily GABAergic and promote sleep by inhibition of the monoaminergic neurons. In turn, the monoamines inhibit the VLPO neurons during wakefulness, thus creating a reciprocal feedback loop, where sleep-promoting and wake-promoting nuclei inhibit each other (Lu et al., 2000; Chou et al., 2002; Gallopin et al., 2000). These mutual inhibition was conceptualized in the flip-flop model by Saper and colleagues (Saper et al., 2001), which will be described in more details in section 1.2.

The VLPO is also connected to other hypothalamic nuclei that are involved in sleep-wake regulation. Inputs from the suprachiasmatic nucleus (SCN) introduce circadian regulation of the VLPO. The VLPO itself inhibits the hypocretin neurons in the lateral hypothalamus (Shiromani et al., 1998). The role of VLPO in sleep is reviewed, for example, by Szymusiak et al. (2001).

Lateral hypothalamic area (LHA)

Particular importance of the lateral hypothalamic area in regulation of sleep was suggested already in 1930 by von Economo (von Economo, 1930). He has proposed that degeneration of neurons in this area is a cause of a sleep disorder narcolepsy, which is characterized by severe disturbances of sleep-wake cycles, inability to stay awake, sleepiness during wakefulness, and other symptoms.

This suggestion was confirmed and explained only recently after the discovery of hypocretin (also called orexin) neurons in the LHA (de Lecea et al., 1998; Sakurai et al., 1998). Diverse experimental studies have shown that narcolepsy is caused by dysfunction of the hypocretin system, i.e. absence of hypocretin neurons, receptors or the neuropeptide itself (Lin et al., 1999; Chemeli et al., 1999; Thannickal et al., 2000; Gerashchenko et al., 2001).

This neuropeptide was simultaneously discovered by two scientific groups (de Lecea et al., 1998; Sakurai et al., 1998). The group of de Lecea has given it a name

hypocretin, due to its structural similarity with hormone secretin. The group of Sakurai has given it the name orexin due to the appetite-stimulating effects of this neuropeptide (orexis is appetite in Greek). Further in the text, for simplicity, I will only use the name hypocretin (hcrt) to refer to this neuropeptide.

Since its discovery in 1998 hypocretin was extensively studied and was found to be involved in diverse physiological functions such as feeding behavior, physical activity and, most importantly for this study, in regulation of sleep and wakefulness (Sakurai, 2007; Sakurai, 2005; Sutcliffe and de Lecea, 2002). The hypocretin-producing neurons in LHA are most active during wakefulness and are virtually silent during NREM and REM sleep (Lee et al., 2005; Mileykovskiy et al., 2005).

Besides of hypocretin, which is released in the synaptic cleft as a neuromodulator, these neurons co-release a classical transmitter glutamate (Rosin et al., 2003). Both substances have depolarizing effect on postsynaptic neurons, binding to different types of receptors. Glutamatergic receptors may be of fast ionotropic or slow metabotropic type, while both hypocretin receptors ($hcrt-r1$, $hcrt-r2$) are metabotropic (Sakurai et al., 1998).

There is only a small number of hypocretin-releasing neurons (about 7000 in the human hypothalamus) but they project literally throughout the whole brain, except of the cerebellum (Peyron et al., 1998; Peyron et al., 2000). Particularly, they excite the monoaminergic and cholinergic nuclei of arousal system and, thereby, indirectly inhibit the sleep-promoting VLPO. Also they send excitatory projections to the thalamus and cortex. They are receiving excitatory input from the SCN and inhibitory input from the sleep-active neurons in the VLPO.

Hypocretin neurons are involved in energy balance by sensing glucose concentrations (Yamanaka et al., 2003). They also receive inputs from the limbic system (Winsky-Sommerer et al., 2004; Sakurai et al., 2005; Yoshida et al., 2006) which are important for emotional arousal. The manifold of connections with sleep-relevant brain areas as well as with autonomous and emotional systems suggests that hypocretin neurons play a crucial role in integrative sleep-wake regulation (for review see Tsujino and Sakurai, 2009; Adamantidis and de Lecea, 2009).

1.1.4 Monoaminergic nuclei

There are several of the sleep-related monoaminergic nuclei, and they are located in different parts of the brain. In the brain stem these are the **noradrener-**

gic locus coeruleus (**LC**) and the **serotonergic dorsal raphe** (**DR**). The **Dopaminergic ventral tegmental area (VTA)** is in the midbrain and the **histaminergic tubero-mammilary nucleus (TMN)** is in the hypothalamus. For details see Hartman, (1974); Jacobs et al., (1984) and Wada et al., (1991).

These diverse nuclei and monoamines have their specific physiological functions but, with regard to sleep-wake regulation they all promote wakefulness (Flicker et al., 1981; Kayama and Koyama, 2003). Accordingly, firing activity of the monoaminergic neurons is high during wakefulness. It is partially suppressed during NREM sleep and completely absent during REM sleep. The sleep-wake activity of these diverse nuclei is described in a number of studies (Hobson et al., 1975; McGinty and Harper, 1976; Trulson and Jacobs, 1979; Puizillout et al., 1979; Cespuglio et al., 1981; Rasmussen et al., 1986; Szymusiak et al., 1989; Sakai et al., 1990; Lu et al., 2006a; Aston-Jones et al., 2007; Ursin, 2008).

Some of the monoamines can have both excitatory and inhibitory effects, depending on the types of postsynaptic neurons and receptors (Bylund, 2007; Ursin, 2008). For example, all of them have inhibitory action on the cholinergic REM-on neurons in laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT). This may explain why the cholinergic neurons are silent when the monoaminergic neurons are firing in the wake and NREM state (McCarley and Hobson, 1975; Lu et al., 2006b). During REM sleep this inhibition is absent and cholinergic neurons are highly active.

With regard to sleep-wake transitions functionally important connections are made between the monoaminergic nuclei and the hypothalamus. Monoaminergic neurons receive excitatory input from the wake-active hypocretin neurons in LHA and are inhibited by the sleep-active neurons in the VLPO. The monoaminergic input to the thalamic neurons helps to keep them in a sensitive state. For the description of the connections of the diverse monoaminergic nuclei see (Ungerstedt, 1971; Freedman et al., 1975; Morrison et al., 1982; Semba and Fibiger, 1992; Luebke et al., 1992; Khateb et al., 1993; Thakkar et al., 1998; Monckton and McCormick, 2002; Kumar et al., 2007; Haas et al., 2008).

Lesions of the monoaminergic nuclei allowed to identify their specific physiological functions during sleep and wakefulness, but they seem to have little impact on overall sleep-wake cycles (Dement et al., 1972; Lidbrink, 1974; Jones et al., 1977; Gerashchenko et al., 2005; Blanco-Centurion et al., 2007). Rather than inducing transitions in the sleep-wake cycles the role of the monoaminergic neurons seems

to be the maintenance of the arousal state (Morgane and Stern, 1975).

1.1.5 Cholinergic nuclei

The cholinergic neurons are located in the **laterodorsal and pedunculopontine tegmental nuclei** of a brain stem (**LDT/PPT**) and in the **basal forebrain** (**BF**) (Armstrong et al., 1983). They are proposed to be of particular importance for generation of REM sleep and thalamocortical desynchronization (Steriade et al., 1991; Jones, 1993). Most of the cholinergic neurons are firing only during REM sleep (REM-on neurons), while some are active during both wakefulness and REM sleep (WAKE/REM-on neurons). In contrast to the monoaminergic neurons they fire preferably in bursting pattern. All of them are suppressed during NREM sleep (Kayama et al., 1992; Siegel, 2000; Szymusiak, 2000).

The cholinergic neurons synthesize acetylcholine and release it at the synaptic terminals having excitatory action on the postsynaptic neurons. They project to the cortex, thalamus and hypothalamus, thereby, promoting REM sleep and wakefulness (Fibiger, 1982; Hobson, 1999). At the same time they are receiving mostly inhibitory inputs from the monoaminergic nuclei, while they are excited by some hypothalamic neurons (LHA) (Gaykema et al., 1991; Steininger et al., 1992; Peyron et al., 1998). The neurons in BF are additionally inhibited by adenosine which accumulates in the extracellular spaces during wakefulness what leads to the promotion of NREM sleep (Thakkar et al., 2003; Arrigoni et al., 2006). The neurons in LDT/PPT are a part of the reticular formation and, apparently, this area was stimulated by Moruzzi and Magoun to induce wake-like EEG in a sleeping animal (Moruzzi and Magoun, 1949, Jones and Beaudet, 1987; Jones and Webster, 1988).

Lesions of the cholinergic nuclei have demonstrated their particular role in regulation of REM sleep episodes, as their number and propensity decreases in correlation with the size of lesion (Deurveilher and Hennevin, 2001; Shouse and Siegel, 1992; Blanco-Centurion et al., 2007). The role of cholinergic neurons in sleep is described in a number of reviews (Steriade and McCarley, 1990; Rye, 1997; Datta, 1997; Hobson et al., 1998; Semba, 1999; Capece et al., 1999; Steriade, 2005).

1.2 Concepts and models of sleep regulation

Diverse brain areas are involved in sleep-wake regulation, as indicated by their activity changes. However, it is not clear how the interaction among them leads to transitions between sleep and wakefulness and what mechanisms trigger these transitions.

The models have been developed to elucidate the essential mechanisms of sleep regulation. They range from descriptive conceptual models to very detailed mechanism-based simulations. The conceptual models are often presented in a form of diagrams (Saper et al., 2001; Saper et al., 2005; Pace-Schott and Hobson, 2002) or described by the mathematical equations (Daan et al., 1984; Jewett and Kronauer, 1999; Achermann and Borbély, 2003; Akerstedt and Folkar, 1997; Mallis et al., 2004; van Dongen, 2004). These models illustrate basic interdependencies between the sleep-related processes and brain areas. The mechanism-based models more specifically focus on the details of the underlying physiological mechanisms (Bazhenov et al., 2002; Hill and Tononi, 2005; Phillips and Robinson, 2007; Diniz Behn et al., 2007; Best et al., 2007; Diniz Behn et al., 2008).

1.2.1 The two-process model

The most influential concept of sleep regulation, on which the majority of recent models of all types are based, is the so called two-process concept that have been developed in 1982 by Alexander Borbély. It does not consider particular brain regions and neurotransmitters but relates sleep-wake cycles to the interaction between a circadian and a homeostatic processes (Borbély, 1982).

The circadian process (C) follows an endogenous, approximately 24 hours rhythm which is coupled to the external environment, especially to the light-dark cycle and social Zeitgebers. Besides of sleep-wake cycles this rhythm can be recognized in diverse physiological functions, such as, body core temperature and hormones release (cortisol, growth hormone, melatonin). The circadian expressiuon of all these rhythms is under the influence of the suprachiasmatic nucleus of the hypothalamus (see section 1.1.3).

The homeostatic process (S) is state-dependent (wakefulness or sleep) and introduces an increase of sleep pressure during wakefulness and its dissipation during sleep. During sleep the time course of the homeostatic process can be derived from

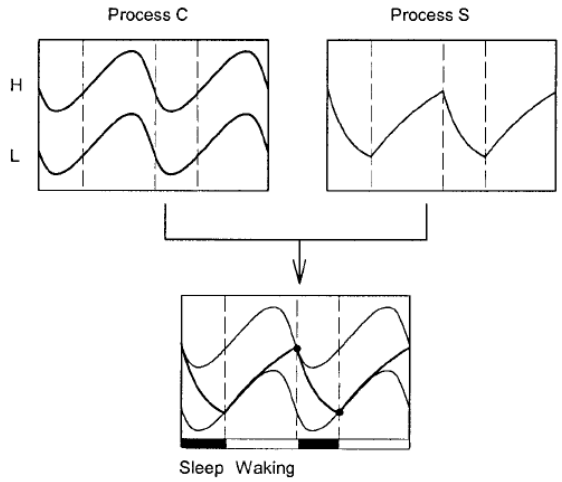

Figure 1.3: Schematic representation of the two-process model of sleep regulation adopted from Achermann (2004). Circadian process C modulates the thresholds H and L. Homeostatic process S rises during waking and declines during sleep. Interaction of the homeostatic process with the thresholds H and L determines the onset and termination of sleep episode.

the changes of slow waves amplitude in the EEG which is also used as a measure of sleep intensity. The longer is the time spent awake above the normal wake time the higher will be the sleep intensity in the following night. The mechanisms of the homeostatic regulation are still unclear.

The two-process concept has been described mathematically by Daan and collegues (1984). Figure 1.3 illustrates its main principles. The circadian process (C) is implemented by two skewed sine functions representing a sleep and a wake threshold (H-high and L-low, respectively). The homeostatic process (S) is modeled in a form of exponential functions. It is accumulating during wakefulness, according to an increasing sleep pressure. During sleep it is decreasing, similar to the dissipating sleep drive. Transitions between wake and sleep states occur when the homeostatic process reaches one of the thresholds of the circadian process.

This model has been successful in illustrating the sleep-wake transitions and predicting the modifications of sleep-wake cycles by various "disturbances", such as day-time nap or shift work (Achermann and Borbély, 2003; Mallis et al., 2004). Extended by an ultradian process the model also accounts for the transitions between different sleep stages (Achermann, 2004).

1.2.2 Extensions of the two-process model

The two-process concept has inspired the development of models of performance and alertness. In addition to the circadian and the homeostatic processes these models use sleep inertia as a third component to account for low alertness immediately after awakening (Åkerstedt and Folkar, 1997; Jewett and Kronauer, 1999; Hursh et al., 2004; Belyavin and Spencer, 2004).

Some data suggested that the sleep inertia is related to the body core temperature, what initiated further extensions of the two-process model, also including temperature modulations (Brown and Luithardt, 1999). These models are widely used by industries, such as air and ground carriers, where alertness of people is a crucial factor (for review see Mallis et al., 2004; van Dongen, 2004).

Such conceptual models are valuable for the understanding of basic principles but they lack an information about the physiological mechanisms. Therefore the physiology-based conceptual models are used to explain and/or investigate the neurophysiological interactions which are relevant for sleep-wake regulation.

1.2.3 The flip-flop concept

As a diversity of brain areas and neurotransmitters is involved in sleep regulation it is important to examine whether the relevant transitions appear due to interactions between the specific nuclei. The key example of such an approach is the flip-flop switch model of Saper et al. (2001, 2005).

It relates the transitions between sleep and wakefulness to the alterations of activity in specific brain nuclei and coupling between them. The major players are the wake-active monoaminergic and hypocretinergic neurons, and the sleep-active GABAergic neurons in the VLPO (Fig.1.4). According to this concept, firing of the monoaminergic neurons results in arousal and wakefulness, while activity of the VLPO neurons leads to sleep. These nuclei are inhibiting each other. The hypocretin neurons serve to stabilize the actual activity state. During wakefulness (a) the depolarizing input from the hypocretin neurons enhances firing of the monoaminergic neurons which strengthens the inhibition of the VLPO neurons. During sleep (b) the VLPO neurons fire and inhibit the monoaminergic nuclei, thereby, relieving their own inhibition.

This model describes the connections between the diverse brain areas and illustrates which of them dominate in the sleep and the wake states. However, it

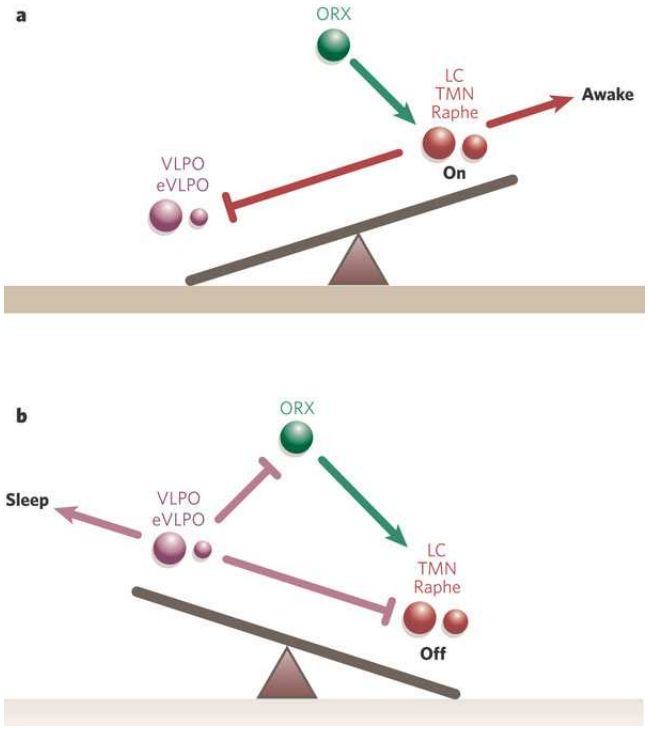

Figure 1.4: Schematic representation of the flip-flop switch model adopted from Saper et al., (2005). During the wake state (a) the hypocretinergic neurons (ORX,green) fire and excite the monoaminergic neurons (red) which are, thereby, inhibiting the VLPO neurons (purple). During sleep (b) The VLPO neurons fire and inhibit the monoaminergic nuclei, thereby relieving their own inhibition. The abbreviations are: ORX - hypocretin/orexin neurons, LC-locus coeruleus, TMN - tuberomammilary nucleus, VLPO - ventrolateral preoptic neurons, eVLPO - extended ventrolateral preoptic neurons.

does not explain what initiates the transitions between the different states and, therefore, does not provide sufficient information to be modeled in a mathematical form. Mathematical models for the understanding of the physiological interactions should be made on a mechanism-based level.

1.2.4 Mechanism-based models

Mechanism-based modeling means mathematical realization of functional interdependencies with variables and parameters that are related to specific physiological processes. Mostly these models simulate neuronal activity on the basis of membrane potentials, currents, ionic conductances, firing frequencies of neurons, concentrations of neurotransmitters and time delays of signal transduction, etc. These models are often focusing on specific features of sleep-wake regulation which are associated with specific brain areas.

The mechanism-based models of sleep regulation can be subdivided into two groups: (1) models which focus on the activity changes in selected brain nuclei or structures during sleep-wake cycles (Bazhenov et al., 2002; Hill and Tononi, 2005), and (2) models which consider the effects of *interactions* between the sleep-related nuclei (Chou, 2003; Diniz Behn et al., 2007; Best et al., 2007; Phillips and Robinson, 2007; Diniz Behn et al., 2008).

The best known examples of the first group are the models of thalamocortical circuits (Bazhenov et al., 2002; Hill and Tononi, 2005). They were developed in order to examine neuronal mechanisms underlying modulations of firing pattern and synchronization of cortical and thalamic neurons at sleep-wake transitions. These models could demonstrate a number of interesting effects, for example, that increase in potassium leak conductances is sufficient to simulate the transition from wakefulness to sleep. However, such models are limited to the selected brain area and do not consider what mechanisms are introducing the relevant changes, e.g. in leak conductances.

The models of the second group try to include the most relevant brain nuclei which regulate sleep-wake cycles and are responsible for the ultradian changes of sleep structure (hypothalamic nuclei, monoaminergic and cholinergic cell groups). For example, the model developed by Diniz Behn et al., (2007) is even capable to reproduce tiny details of the mice sleep structure, including short and long wake bouts. An extended model could explain the experimentally observed effect that

hypocretin is essential for long but not for short (<1 min) wake bouts (Diniz Behn et al., 2008). These effects were related to the time-dependent hypocretin-mediated increase of the monoaminergic inhibition of sleep-promoting neurons in VLPO.

Another example is the quantitative model of sleep-wake dynamics based on the physiology of the arousal system (Phillips and Robinson, 2007). The major advantage of this model, comparing to others, is that its parameters are not free but correspond to physiological observables. The model replicates the human sleep-wake cycle, with physiologically reasonable voltages and firing rates in the considered brain areas, i.e. VLPO, monoaminergic and cholinergic nuclei. Furthermore, the model develops hysteresis with a region of bistability in which the transitions between sleep and wake states occur. The narrowing of this bistability zone results in frequent transitions between the states of sleep and wake, what is proposed to resemble narcolepsy.

However, irrespective of how detailed the mechanism-based models are, they all require implementation of the circadian and the homeostatic processes to induce the periodic transitions between sleep and wakefulness. The circadian mechanisms are well studied and were realized in diverse conceptual and mechanism-based models (Kronauer et al., 2007; Goldbeter, 2002). As for the homeostatic process, it is usually implemented not on the mechanism-based level, but as an explicit function in a form similar to that of the conceptual two-process model. This is to a big extend related to a little and sometimes contradictory knowledge about the physiological background of the homeostatic sleep process.

1.3 Sleep homeostasis: state of the art

The term sleep homeostasis, as proposed by Borbély, mostly refers to the NREM sleep homeostasis. REM sleep seems to be a very specific state which, obviously, is completely different from all other sleep states and, accordingly, is governed by its own, particular rules (Brunner et al., 1990). Therefore, here and further in the text, whenever the term sleep homeostasis is used, it refers to the regulatory processes only of NREM sleep.

The time course of the homeostatic process in the two-process model (Daan et al., 1984) has been derived from the slow waves activity in sleep EEG which

serves as a marker of NREM sleep intensity and homeostatic changes (Borbély et al., 1981). The amplitude of slow delta waves continuously declines in the course of sleep. The amplitudes of delta waves during sleep are correlated with the time spent awake. They are higher and the sleep phase is longer when the duration of the preceding awake time is prolonged (Borbély et al., 1981; Dijk et al., 1993; Aeschbach et al., 1996). In this way, sleep deprivation is counteracted during the following night by the enhanced sleep intensity and prolonged time of sleep (sleep rebound) (Borbély and Achermann, 2005). A physiological marker of sleep homeostasis can also be tracked during wakefulness by measuring the power of theta-band of the waking EEG, which increases during prolonged wakefulness (Cajochen et al., 1995).

Although the EEG markers of NREM sleep homeostasis are well established, the chemical and neuronal mechanisms underlying them are not well understood. Diverse neurotransmitters, hormones and prostoglandines, including norepinephrine, serotonin, histamine, prostoglandine D2, adenosine, hypocretin and others are contributing to regulation of sleep and wakefulness.

Various brain areas change their activity along the sleep-wake transitions. Some are assumed to be driven by the other nuclei, while others are more likely to regulate the transitions between the sleep and the wake states. Among the nuclei that are believed to regulate the sleep-wake cycles are the diverse nuclei of the hypothalamus (Saper et al., 2005). Also, most of the body's autonomous and homeostatic functions, including blood pressure, body temperature, fluid balance and others are controlled by the hypothalamus (Kandel et al., 1991). The hypothalamus, therefore, appears to be a good candidate in search for homeostatic sleep functions.

In the hypothalamus, besides of the SCN with its circadian pacemaker neurons, there are at least two more nuclei which significantly influence the temporal structure of sleep-wake cycles. These are the ventrolateral preoptic nucleus (VLPO) with sleep-active GABAergic neurons (Sherin et al., 1996) and the lateral hypothalamic area (LHA) with wake-active hypocretin neurons (Lee et al., 2005; Mileykovskiy et al., 2005). Lesions of the VLPO lead to insomnia in a strength proportional to the size of the lesion (Lu et al., 2000). Lack of hypocretin, its receptors or hypocretin-producing neurons leads to a sleep disorder narcolepsy, which is characterized by severe disturbances of sleep-wake cycles, high somnolence, spontaneous transitions to sleep and other symptoms (Thannickal et al.,

2000; Gerashchenko et al., 2001; Peyron et al., 2000; Chemeli et al., 1999; Lin et al., 1999). However, it is still an open question whether and how the activity of these nuclei can also serve as a homeostatic sleep process.

1.3.1 The adenosine-theory of sleep homeostasis

Possible contribution of the sleep-active VLPO neurons to a homeostatic process is often discussed in context with its activation by adenosine (Basheer et al., 2004; Landolt, 2008). The adenosine-based theory of sleep homeostasis has attracted much attention. Adenosine is a ubiquitous nucleoside which can, principally, appear in any cell. The idea of adenosine-based homeostasis has developed from the hypothesis that one of the functions of sleep is energy restoration. Therefore, it was suggested that adenosine, as a byproduct of energy metabolism, may act as a homeostatic regulator of energy in the brain, increasing in concentration during wakefulness and dissipating during sleep (Chagoya de Sanchez et al., 1993; Benington and Heller, 1995). Such increase of adenosine concentration can be expected because extracellular concentration of adenosine increases with increased neural activity and metabolism, and wakefulness has 30% higher metabolic rate than NREM sleep (McIlwain and Pull, 1979; Van Wylen et al., 1986; Tobler and Scherschlicht, 1990; Meghji, 1991; Minor et al., 2001; Maquet et al., 1992; Madsen, 1993).

Remarkably, increasing adenosine concentration during wakefulness could not be found in all brain regions. During normal wake times the most pronounced increase has been seen in the cholinergic basal forebrain (Strecker et al., 2000) and to a lesser extend in the preoptic hypothalamic region and cerebral cortex (Porkka-Heiskanen et al., 2000; Alam et al., 1999; Huston et al., 1996). During sleep deprivation enhanced adenosine levels have been seen only in the cholinergic basal forebrain. In contrast, in the VLPO region sleep deprivation results in the decrease, rather than increase, of adenosine concentration (Porkka-Heiskanen et al., 2000; Basheer et al., 1999). Due to this and other data it was concluded that VLPO does not accumulate the need for sleep, but is rather being under the influence of homeostatic factors of other origin (Saper et al., 2005).

Nevertheless, other observations seem to fit quite well to the adenosine-theory of sleep homeostasis. For example, the fact that injections of adenosine in the cholinergic BF, LDT/PPT and hypocretinergic LHA as well as near the VLPO

induce sleepiness (Dunwiddie and Worth, 1982; Virus et al., 1983; Ticho and Radulovacki, 1991; Portas et al., 1997). It has been shown that adenosine suppresses activity of the wake-promoting cholinergic and hypocretinergic neurons by a direct inhibition (Rainnie et al., 1994; Liu and Gao, 2007), while it activates sleep-promoting GABAergic VLPO neurons by inhibition of inhibitory connections from the wake-promoting neurons (Chamberlin et al., 2003; Morairty et al., 2004; Gallopin et al., 2005; Methippara et al., 2005). The activation of the VLPO neurons was proposed to be primarily realized via the inhibition of the cholinergic neurons in BF which indirectly inhibit VLPO (for review see Basheer et al., 2004; McCarley, 2007; Landolt, 2008). Furthermore, the well known wake-promoting effect of caffeine can be explained by its action as an adenosine antagonist (Fredholm et al., 1999). Systemic administration of caffeine prolongs the sleep latency and reduces slow wave sleep what indicates impaired sleep homeostasis (Landolt et al., 1995; Landolt et al., 2004; Drapeau et al., 2006; James and Keane, 2007).

All these findings demonstrate the importance of adenosine in regulation of sleep and its possible contribution to the homeostatic process. However, some of the recent studies suggest that this concept is not complete and that other mechanisms may, at least additionally, be involved.

As it was described above the BF is an area with the strongest increase of adenosine concentration during wakefulness. Therefore, adenosine-related inhibition of BF cholinergic neurons was proposed as one of the mechanisms of homeostatic regulation of sleep. Blanco-Centurion and colleagues (2006) have studied this hypothesis by examining sleep intensity it rats with lesion of the cholinergic basal forebrain. Remarkably, in these BF lesioned rats, the increase of sleep intensity after sleep deprivation is of the same extent as in control rats. Accordingly, the accumulation of adenosine in the cholinergic BF, which does not occur in the lesioned rats, cannot be responsible for the increasing sleep intensity after sleep deprivation. Other experiments of the same group have shown that even triple lesions when, apart from the BF, also the TMN and the LC have been destroyed, do not affect the total wake time. Obviously, these centers, although they may promote wakefulness, do not significantly contribute to homeostatic regulation.

Furthermore, Stenberg with colleagues (2003) have shown that mice without A1 adenosine receptors, which are proposed to be crucial for the sleep-promoting adenosine effects, show neither alterations in sleep-wake cycles nor changes in the rebound sleep intensity compared to control. Indeed, this may be explained by

a reorganization of homeostatic mechanisms in the absence of A1 receptor, but can also indicate that other mechanisms are more important for homeostatic sleep regulation.

1.3.2 Contribution of hypocretin to the control of sleep and wakefulness

The neuropeptide hypocretin is not such a ubiquitous substance as adenosine but is a co-transmitter of an only small population of neurons in the lateral hypothalamic area (LHA) (de Lecea et al., 1998; Sakurai et al., 1998). Release of hypocretin is high during wakefulness and is activity-dependent (Kiyashchenko et al., 2002). Therefore, concentration of hypocretin in the cerebrospinal fluid (CSF) increases during a day and decreases during a night (Salomon et al., 2003; van den Pol, 1999; Fujiki et al., 2001; Yoshida et al., 2001), what makes a similarity to a concept of accumulation of "sleep substance", as with adenosine.

The hypocretin neurons have excitatory projections to virtually all arousal brain areas (Peyron et al., 1998; Date et al., 1999; Nambu et al., 1999) and, thereby, are proposed to play a major role in maintenance of wakefulness (for review see Sakurai, 2007; Sutcliffe and de Lecea, 2002). Intracerebrovascular injection of hypocretin lengthens the total wake time (Ida et al., 1999) and intravenous and/or nasal administration improves performance of sleep deprived subjects (Deadwyler et al., 2007). Application of hypocretin antagonist, almorexant, was shown to induce sleepiness and increase amount of both NREM and REM sleep (Brisbare-Roch et al., 2007).

Hypocretin neurons are also involved in sensing body's external and internal environments, including energy state and emotional stimuli, what helps them to adjust sleep-wake cycles according to the actual body state and other homeostatic features (Sakurai et al., 2005; Yamanaka et al., 2003; Yoshida et al., 2006).

The impact of hypocretin on sleep-wake control, especially for the maintenance of wakefulness, is much more clearly demonstrated than for any other substance. However, the question is whether it also can be a homeostatic factor, i.e. a substance that accumulates sleep pressure during wake state.

Chapter 2

CONCEPT

A novel concept for sleep homeostasis

Considering the above mentioned facts and the lack of a comprehensive, mechanism-based explanation of sleep-wake transitions, a novel concept of a homeostatic process has been developed. The idea is that the homeostatic alterations of sleep or wake drives must not necessarily result from the accumulation and decline of specific substances but can also arise from different, state-dependent mechanisms. The proposal is that homeostatic changes are directly related to the neuronal dynamics, specifically to activity-dependent alterations of synaptic transmission.

The concept is based on the fact that not only lack of hypocretin neurons but also lack of hypocretin itself or its receptors leads to significant disturbances of sleep-wake cycles such as those observed in narcolepsy. This means that sleep pressure develops due to reduced availability or weakened synaptic efficacy of hypocretin (lack of hypocretin or its receptors). This even might happen when the neurons and neuronal circuits are intact and the main neurotransmitter of hypocretin neurons - glutamate (Rosin et al., 2003) is fully operating. Similar effects might occur during the natural sleep-wake cycles.

During wakefulness, when wake-promoting hypocretin neurons are tonically firing, hypocretin synaptic transmission may decrease, what results in increased sleep pressure and transition to sleep. During sleep the synaptic transmission may recover. Such state-dependent changes of synaptic activity can result from diverse mechanisms. For example, it can be expected that the amount of hypocretin which is released into the synaptic cleft during the ongoing firing in the wake state cannot

be fully compensated. Neuropeptides, like hypocretin, are not re-uptaken but disappear from the cleft by degradation or diffusion. Therefore, the availability of such co-transmitters may decrease easier than that of classical transmitters like glutamate. Another well known mechanism that leads to reduced synaptic efficacy is the internalization of receptors in response to ongoing stimulation by receptor-agonists. In the silent state, the synaptic efficacy can slowly increase due to refilling of the presynaptic content or by receptor up-regulation, respectively.

The transitions between the active wake and silent sleep states of hypocretin neurons can be related to the alterations of the synaptic efficacy assuming reciprocal excitatory feedback connections between the hypocretin neurons and other excitatory neurons, e.g. local glutamate interneurons. It is proposed that these experimentally well established connections are required to keep the hypocretin neurons in a firing state (Li et al., 2002). Firing of the hypocretin neurons activate local interneurons which, in turn, keep the hypocretin neurons in a firing state via reciprocal excitatory projections. This is the way how sustained activity can be achieved whenever the one or the other type of neurons have been sufficiently excited for activation of their reciprocally connected partners which, together, constitute a positive feedback loop.

The question is, how this positive feedback loop is interrupted, i.e. how such reciprocal excitatory circuits can go from sustained firing to silence. This is the point where the activity-dependent alterations of the synaptic efficacy of the co-transmitter hypocretin come into play. The idea is that the hypocretin neurons, in addition to their main transmitter glutamate, need a certain contribution of the co-transmitter hypocretin to keep the interneurons in a firing state. When this is no longer possible because of impaired hypocretin effects, the cessation of firing of the interneurons will bring the complete reciprocal circuits into a silent state. Once firing has ceased it will need some recovery time to reintroduce sustained impulse generation. This can be achieved by any external stimulus which is strong enough to introduce firing in one the neuronal populations which can activate the other one. Such stimuli may come from circadian pacemaker neurons or from a sensory input, e.g. form an alarm clock.

These state-dependent alterations of the synaptic efficacy and firing activity of hypocretin neurons, of course, will have impact at all their synaptic terminals in the manifold of brain areas to which they project. Most important effects with regard to sleep-wake cycles can be expected from the direct or indirect projections

of hypocretin neurons to the thalamus. The thalamic neurons are considered to play a major role as a gate for sensory information processing. This is of particular importance for the transition from a conscious wakefulness to an unconscious sleep state.

The thalamic neurons are going from unsynchronized single spike generation in the wake to synchronized burst discharges in the sleep state (McCormick and Feeser, 1990). Such transitions can be achieved in different ways, for example, by alterations of the leak conductances as modeled by Hill and Tononi (2005). The easiest way to bring bursting neurons into a tonic firing state, which typically is used in electrophysiological experiments, is the injection of a depolarizing current. These alterations of the spiking pattern seem to be sufficient to bring the neurons out of a synchronous into asynchronous state of impulse generation. The transitions between different impulse patterns and synchronization states as a function of current injection may simply develop due to the particular intrinsic properties of thalamic neurons (Postnova et al., 2007b).

Such tonic-to-bursting transitions in the activity of thalamic neurons were shown to appear as a results of depolarization due to extracellular application of hypocretin (Kolaj et al., 2007). It can be expected that the same transitions can be achieved by a synaptic current input from hypocretin neurons. When the hypocretin neurons are firing, their depolarizing synaptic input will keep the thalamic neurons in an unsynchronized tonic firing state. Without this depolarizing input, when the hypocretin neurons are silent, the thalamic neurons may go into a synchronized bursting mode.

In this way, the proposed mechanisms of homeostatic alteration of the synaptic hypocretin efficacy may not only account for the sleep-wake related transitions of the impulse activity of hypocretin neurons but also for the alterations of impulse pattern and synchronization states of thalamic neurons.

This concept has been translated into a mathematical model to examine whether and how the proposed transitions can be achieved and to elucidate the functionally relevant dynamics. Both the concept and the model will be published in Journal of Biological Rhythms (Postnova et al., in print b) and will further be described here in the MODEL and RESULTS chapters. The modeling approach and its physiological background will be described in details in the next section (METHODS).

Chapter 3

METHODS

To mathematically realize the above described concept on the level of neurons and synapses a conductance-based approach was used. This approach was developed in 1952 by Alan Lloyd Hodgkin and Andrew Fielding Huxley who have combined experimental and modeling techniques to explain the mechanisms of generation of action potential (Hodgkin and Huxley, 1952). A major advantage of this method is that the variables and parameters of the model can, in principal, be measured in electrophysiological experiments and their dynamics follow physiological rules.

This chapter presents the general equations of the conductance-based approach. It describes the assumptions that are made to bring such complicated systems as neurons and synapses down to a set of mathematical equations without loosing the functionally relevant properties.

3.1 Membrane equation

The lipid bilayer of membrane can be interpreted as an electrical capacitor. It is charged and discharged by the ionic currents I_i flowing through the ion channels in the membrane. The ion channels are represented as conductances g_i for the corresponding type of ions. Altogether the neuron can be considered as an equivalent circuit, as shown in the Figure 3.1.

According to the Kirchhoff's current law the total current in electrical circuit equals to zero. Therefore, the current in the membrane capacitor I_C equals the sum of all the ionic currents flowing through the membrane.

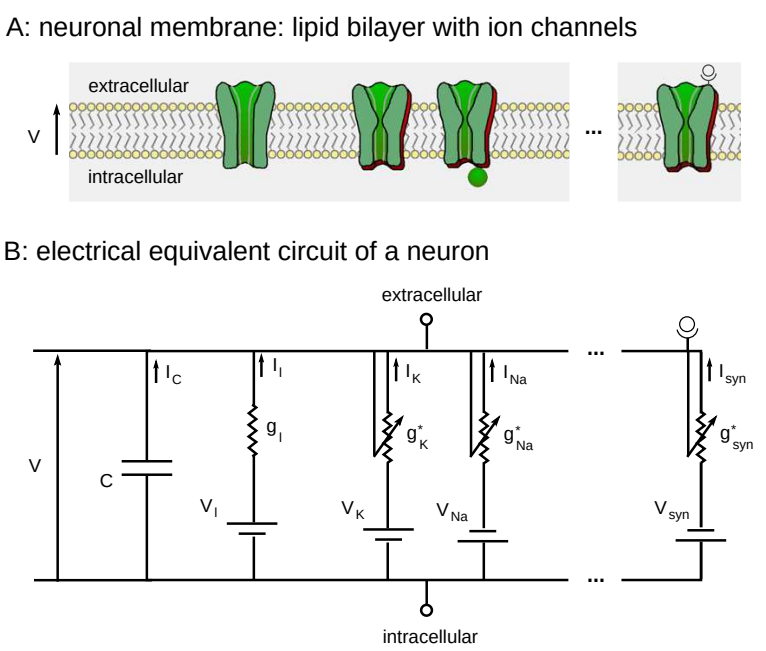

Figure 3.1: A: Lipid bilayer of a neuronal membrane with different types of ion channels in it. From left to right: passive leak, potassium, sodium and transmitter-gated channels. B: Schematic representation of a neuronal membrane according to the conductance-based approach. The capacitor C is charged by all the currents in the circuit (I_l, I_{Na}, I_K, I_{syn}, etc). I_l is a leak current flowing through the constant conductance g_l with equilibrium potential V_l. I_{Na} and I_K are flowing through the voltage-dependent conductances g_{Na} and g_K respectively. Current I_{syn} is a synaptic transmitter-gated current, which appears due to coupling with other neurons. The sum of the currents equals to the capacitive current I_C.

$$I_C = -\Sigma I_i. \tag{3.1}$$

The capacitive current is defined as a time rate of change of charge: $I_C = dQ/dt$. The charge Q, on the other hand, can be written as a product of the membrane capacitance C and voltage: $Q = CV$. Thus, the Eq.3.1 can be modified into the differential equation for membrane voltage:

$$C\frac{dV}{dt} = -\Sigma I_i. \tag{3.2}$$

The original Hodgkin-Huxley model included three types of ionic current: leak I_l, sodium I_{Na} and potassium I_K. However, this modeling approach allows extensions by any other types of currents, including synaptic and externally applied currents, which are simply added to the right side of the membrane equation.

$$C\frac{dV}{dt} = -I_l - I_{Na} - I_K - I_{syn} - I_{external} - ... \tag{3.3}$$

3.2 Implementation of membrane currents

The ionic currents, in a general form, are calculated as the product of the ionic conductance g_i and the driving force. The driving force is given by the difference between the actual membrane potential V and the equilibrium potential of the ions that contribute to the current V_i.

$$I_i = g_i(V - V_i). \tag{3.4}$$

The equilibrium potential for a specific type of ions is equal to the Nernst potential V_N and can be calculated according to the Nernst equation:

$$V_i = V_{N_i} = \frac{RT}{zF} ln \frac{[i]_{ext}}{[i]_{int}}, \tag{3.5}$$

where R is the gas constant, T is the absolute temperature (in degrees Kelvin),

z is the valence of the ion, F is the Faraday constant, and $[i]_{ext}$ and $[i]_{int}$ are the concentrations of the ion in the extracellular and intracellular space, respectively.

For unspecific ion channels, through which different types of ions can pass, the equilibrium potential is a mixture of the Nernst potentials that also depends on the conductivity of the channels for the different ions. In this case the equilibrium potential is calculated according to the Goldman-Hodgkin-Katz equation, which in terms of the conductances can be reformulated in the following way:

$$V_i = \frac{\Sigma g_i V_{N_i}}{\Sigma g_i}. \tag{3.6}$$

3.2.1 Leak currents

Without voltage, synaptically or otherwise gated currents any neuron will have a constant membrane potential that is exclusively determined by the leak currents. Although the leak currents are carried by diverse ions that are flowing through different channels with different conductances, for practical reasons, they are mostly comprised in a single term:

$$I_l = g_l(V - V_l). \tag{3.7}$$

The leak conductance g_l as well as the equilibrium potential of the leak current V_l are mainly determined by K^+ ions because they contribute with the highest leak permeability, as well as by the Na^+, Cl^- ions and, to a less extent, Ca^{2+} ions.

The value of g_l, especially in comparison to the conductances of other currents, is an important parameter which determines the stability of the resting membrane potential against voltage- and synaptically-induced currents. Changes of the leak conductance, therefore, can have significant impact on the neuron's excitability. However, for most situations they can be considered constant.

The equilibrium potential V_l sets the voltage that the neuron achieves at rest (typically around $-70mV$). Its position in relation to the activation curves of voltage-gated currents is an additional important factor with regard to the neuron's excitability.

3.2.2 Voltage-gated currents

The voltage-dependent currents can be calculated in, principally, the same form as the leak current.

$$I_i = g_i^*(V - V_i). \tag{3.8}$$

However, the ionic conductances, as indicated by the $*$, are not constant but change as a function of the membrane voltage and exhibit functionally important time delays.

The best known examples of voltage-gated currents are the sodium (Na) and potassium (K) currents that are required for generation of action potential (AP). These currents have been originally analyzed by Hodgkin and Huxley for the AP of the squid giant axon. In their ingenious work, Hodgkin and Huxley have related these ionic currents to activation (opening) and inactivation (closing) of ion channels which can go through different steps as a function of voltage and time. Therefore, the factual conductance is calculated by

$$g_i^*(V,t) = g_i a_i^x(V,t) b_i^y(V,t), \tag{3.9}$$

where g_i is the maximum conductance which is achieved when all of the specific voltage-gated channels are open. The fraction of open ion channels is given by the activation and inactivation variables a and b (normalized to 1) which are determined by different voltage- and time-dependent gating rules. The power values x and y shall account for the different states through which the ion channels are going during activation and inactivation. This is described in details in the 1952 work of Hodgkin and Huxley and in many subsequent papers but shall not further be specified here.

In any case, the result is an approximately sigmoid voltage dependence of the ionic conductance. This perfectly complies with experimental recordings in which the voltage dependencies of ionic conductances can be measured. In contrast, the gating variables are difficult to record.

Accordingly, actual modifications of the Hodgkin-Huxley-type models are often built up on a simplified approach where the voltage dependencies of the activation and inactivation variables are implemented by explicit sigmoid functions:

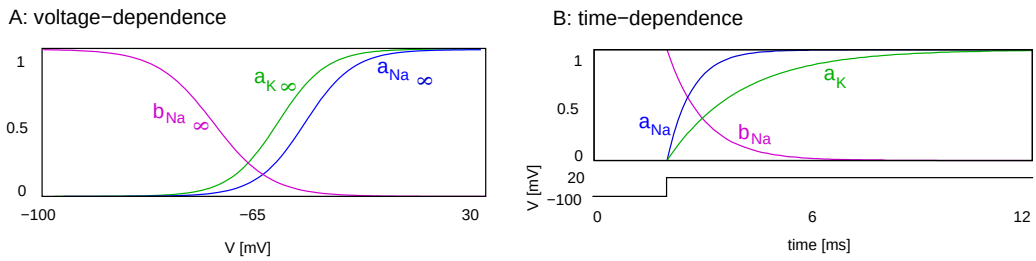

Figure 3.2: A: Typical voltage dependencies for steady state activation (a_{Na_∞}, a_{K_∞}) and inactivation (b_{Na_∞}) for sodium and potassium currents. B: corresponding time dependencies of the activation a_{Na}, a_K and inactivation b_{Na}.

$$a_{i_\infty} = \frac{1}{1 + exp(-s_{a_i}(V - V_{a_{0i}}))}, \tag{3.10}$$

$$b_{i_\infty} = 1 - \frac{1}{1 + exp(-s_{b_i}(V - V_{b_{0i}}))}. \tag{3.11}$$

Here, s_i is the slope of the sigmoid curve and V_{0i} is the half-activation (inactivation) potential that are achieved when half of the ion channels are opened (closed).

Beyond the voltage dependencies, dynamically important properties are introduced by the different time delays of current activation and inactivation. It is easy to understand that the generation of action potentials is only possible because of the faster activation of Na than K currents - irrespective of the voltage dependencies. Typical curves for voltage and time dependencies for activation of both sodium and potassium currents and for inactivation of sodium current are shown in Figure 3.2.

In the original Hodgkin-Huxley approach, the time delays follow complicated voltage dependencies that were introduced to achieve the best fit of the model to the experimental data under investigation, i.e. the shape of the action potential (AP). In the actual modeling approach, the precise shape of the AP is of minor interest. Major interest is laid on the generation of AP by the neurons, their firing rates and impulse patterns.

Accordingly, in simplified versions, the voltage dependencies of the activation

and inactivation time constants are mostly neglected. The time constants are, indeed, constant at all voltages. Moreover, the activation and inactivation variables a_i and b_i follow the voltage-dependent changes a_{i_∞} and b_{i_∞} with simple first order time delays (τ_{a_i} and τ_{b_i}).

$$\frac{da_i}{dt} = \frac{a_{i_\infty} - a_i}{\tau_{a_i}}, \tag{3.12}$$

$$\frac{db_i}{dt} = \frac{b_{i_\infty} - b_i}{\tau_{b_i}}. \tag{3.13}$$

An additional simplification can be introduced when also the time-dependent inactivation is neglected. This is justified because closing of ion channels, anyhow, appears due to the inherent voltage dependencies. Altogether, the current equation can be written in a significantly simplified form:

$$I_i = g_i a_i (V - V_i), \tag{3.14}$$

where g_i still is the maximum conductance, while a_i comprises the above described voltage (Eq. 3.10) and time dependencies (Eq. 3.12).

3.2.3 Synaptic currents

The connections between neurons in neuronal networks can be made in different ways: via gap junctions or via chemical synapses. In the conductance-based approach, the synaptically introduced currents simply appear as additional terms in the voltage equations (Eq. 3.2 or 3.3). However, their structure is completely different depending on the type of connection.

Gap junctions are made by connexons (2 for each gap junction) that directly connect the cells. They constitute an unspecific pore of mostly constant conductance through which ions can diffuse in both directions (from one neuron to the other) according to the voltage difference between the neurons.

The gap junction current of an individual neuron connected with some number n of the neighbored neurons can be described as the sum of the diffusive/electroto-

nic currents:

$$I_{gj} = \Sigma g_{gj_j}(V - V_j), \tag{3.15}$$

for $j = 1$ to n. Here $(V - V_j)$ is the actual voltage difference between the individual neuron and one of its neighbors j, and g_{gj_j} is the conductivity which is made up by the gap junctions between the two neurons.

Chemical synapses make unidirectional connections between a pre- and a postsynaptic neuron without direct electrical contacts. In these cases, an action potential in the presynaptic neuron leads to the release of chemical transmitters at its synaptic terminals. The transmitter diffuses through the synaptic cleft to the postsynaptic membrane (soma, dendrites or synaptic endings) where it binds to specific receptors what leads to the opening or closing of ion channels. The current is determined by the conductance of these transmitter-gated channels and by the driving force of the ions which can pass through these channels.

$$I_{tg_{post}} = g_{tg} a_{tg}(V_{post} - V_{tg}), \tag{3.16}$$

where V_{post} is the membrane potential of the postsynaptic neuron and V_{tg} is the equilibrium potential of the transmitter-gated channels. When these channels allow different ions to pass, the equilibrium potential is determined by the conductivity for the different ions and can be calculated in the same way as the equilibrium potential for the leak currents. Different from the gap junction currents, the driving force does not depend in any way on the voltage of the presynaptic neuron but is only determined by postsynaptic properties.

The synaptic conductance is again calculated as the product of a maximum conductance g_{tg} (when all the transmitter-gated channels are open) and an activation variable a_{tg} which denotes to the fraction of the actually open ion channels.

This is, formally, the same structure as for voltage-gated currents. In this case, however, the activation variable does not depend on the membrane potential but on the concentration of transmitters in the synaptic cleft which is determined by its release and elimination. Assuming that the opening and closing of transmitter-gated channels is directly proportional to the transmitter concentration we, again, can write the equation for the activation variable of transmitter-gated channels in

the same form as for voltage-gated channels:

$$\frac{da_{tg}}{dt} = \frac{a_{tg_{rel}} - a_{tg}}{\tau_{tg}}. \tag{3.17}$$

The variable a_{tg}, likewise, stands for the fraction of activated ion channels as well as for the transmitter concentration. Accordingly, $a_{tg_{rel}}$ reflects the transmitter release which directly determines the activation. The time constant τ_{tg} essentially accounts for the time delays of transmitter elimination. The time delays of transmitter release can be adjusted by the value of $a_{tg_{rel}}$.

The function $a_{tg_{rel}}$ formally corresponds to a_∞ in the equation of voltage-dependent currents and is modeled in the same way, i.e. by a sigmoid function:

$$a_{tg_{rel}} = \frac{1}{1 + exp(-s_{spike}(V_{pre} - V_{spike}))}. \tag{3.18}$$

However, in case of synaptic transmission it has a completely different meaning comparing to the voltage-gated currents. This equation connects the pre- and postsynaptic effects in determining the transmitter release and, hence, the postsynaptic current activation in response on a presynaptic action potential. Accordingly, the sigmoid function has an extraordinarily steep, almost threshold-like voltage dependence which is given by s_{spike}. V_{pre} is the presynaptic membrane potential and V_{spike} is the half-activation potential for transmitter release. It is set to a high potential value (e.g. at around $-20mV$) which makes sure that an action potential is required for transmitter release and postsynaptic current activation. Physiologically, this function may reflect the activation of presynaptic, high threshold Ca current which leads to the fusion of synaptic vesicles with the presynaptic membrane and the release of transmitters.

Altogether, the manifold of pre- and postsynaptic processes which are involved in synaptic transmission can be comprised in simplified, but physiologically justified, model equations with a similar form as for voltage-gated currents.

It is the major advantage of this conductance-based approach that it allows to implement in a comparably simple, additive way a diversity of ionic currents, from leak to voltage-gated, gap junction and transmitter-gated currents, and that all these currents can be modeled with physiologically appropriate variables and

parameters.

These are the basic functions on which a conductance-based model can be built up and which will be used for the model of sleep-wake cycles - with one significant extension according to its specific function, which is homeostatic synaptic plasticity.

Computationally the model system was realized using the programming language "C" and calculated by the well known numeric method of Runge-Kutta (Fortsythe et al., 1977; Press et al., 1988; Butcher, 2003).

Chapter 4

MODEL

The basic idea for the mechanism-based modeling of the homeostatic processes comes from the experimental data that suggest that the firing of hypocretin neurons during wakefulness is sustained by reciprocal excitatory connections with other neurons, including local glutamate interneurons in the LHA (Li et al., 2002). Weakening of these feedback loops results in decrease of firing activity, what corresponds to a reduction of wake drive, i.e. building-up of sleep pressure. The proposal is that this weakening is a direct result of reduced synaptic efficacy due to the ongoing firing of the neurons during wakefulness. During the silent sleep state the synaptic efficacy recovers, thus, facilitating the reactivation of the neurons, i.e. their transition to firing and awakening.

The thalamic neurons are assumed to be under the control of the hypothalamic centers of sleep-wake regulation, including the hypocretin neurons in the LHA with direct and/or indirect projections, e.g. via monoaminergic nuclei and/or the ascending reticular activation system (ARAS). The excitatory hypocretin effects during wakefulness can help to keep the thalamic neurons in an asynchronous tonic firing state, while the lack of hypocretin input during sleep facilitates the transition of thalamic neurons to burst discharges and synchronization.

The model is built up with a minimal set of only four neurons (Fig.4.1), because major emphasis is laid on the synaptic connections and their state-dependent alterations. Two neurons are constituting the feedback loop for homeostatic sleep-wake regulation, one representing the hypocretin neurons in the lateral hypothalamus and another one the local glutamate interneurons. All inputs from other nuclei, e.g. from the circadian pacemaker in the SCN, are comprised in exter-

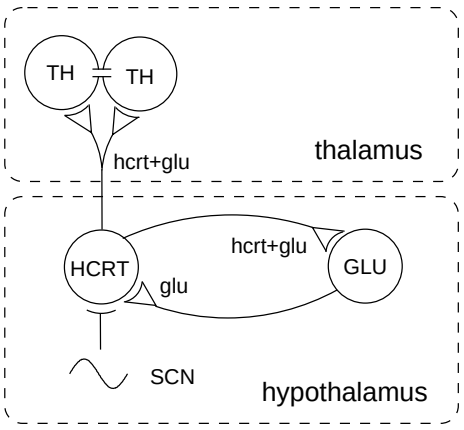

Figure 4.1: Schematic representation of the model of sleep-wake cycles. The hypocretin neuron (HCRT) is synaptically innervating the glutamate (GLU) and thalamic (TH) neurons with release of glutamate (glu) and co-release of hypocretin (hcrt). The synaptic connection from the glutamate neuron to the hypocretin neuron is solely made via glutamate release. The connection between the thalamic neurons is realized via gap junction. The hypocretin neuron additionally receives circadian input from the SCN in a form of a skewed sine wave.

nal currents to the hypocretin neuron. A minimal set of two additional neurons is needed to demonstrate thalamic synchronization and desynchronization. For simplicity, these two thalamic neurons are electrotonically coupled via gap junctions. All other connections are made by chemical synapses. As a functionally most important feature, synaptic transmission of the hypocretin neuron includes a co-transmitter, the neuropeptide hypocretin, which is assumed to introduce the relevant homeostatic mechanisms.

The neurons and their synaptic connections are implemented with a conductance-based approach as described in the previous chapter.

4.1 Membrane potentials

The neuronal membrane equations are given in the general form as described above by the Eq.3.3. The membrane equations of different neurons can include different current terms. All of the modeled neurons have the terms for leak current I_l and spike-generating Na and K currents I_{Na} and I_K. The thalamic neurons possess two additional terms for subthreshold currents to allow the generation of membrane potential oscillations and burst discharges. Additional, functionally

most important effects arise from specific types of synaptic currents.

Everywhere throughout the equations upper-case letters (HCRT, GLU and TH) are used to indicate the neurons, while lower-case letters (hcrt and glu) refer to the transmitters.

The membrane equations of the hypothalamic neurons are given by:

$$C\frac{dV_{HCRT}}{dt} = -I_{l_{HCRT}} - I_{Na_{HCRT}} - I_{K_{HCRT}} - I_{glu_{HCRT}} + I_{circadian} + I_{ext_{HCRT}}, \tag{4.1}$$

$$C\frac{dV_{GLU}}{dt} = -I_{l_{GLU}} - I_{Na_{GLU}} - I_{K_{GLU}} - I_{glu_{GLU}} - I_{hcrt_{GLU}}. \tag{4.2}$$

These neurons are simulated with the minimal possible set of voltage-dependent currents. The relevant dynamics arise from their reciprocal synaptic connections. The hypocretin neuron receives synaptic current input from the glutamate neuron $I_{glu_{HCRT}}$. Vice versa, the glutamate neuron receives glutamatergic current input from the hypocretin neuron $I_{glu_{GLU}}$. This synapse, however, additionally releases the co-transmitter hypocretin which is considered by a second synaptic current term $I_{hcrt_{GLU}}$.

The hypocretin neuron possesses an additional term for the injection of external currents, which also allows to simulate, in a simplified way, a compound synaptic current input from the circadian pacemaker neurons in the suprachiasmatic nucleus.

The membrane equations of the two thalamic neurons are identical:

$$C\frac{dV_{TH}}{dt} = -I_{l_{TH}} - I_{Na_{TH}} - I_{K_{TH}} - I_{Na,p_{TH}} - I_{K,Ca_{TH}} - I_{gj_{TH}} - I_{glu_{TH}} - I_{hcrt_{TH}} + I_{ext_{TH}} + I_{noise_{TH}}. \tag{4.3}$$

These neurons, according to experimental data, should be able to change their activity pattern from single spike (tonic firing) to grouped discharges (bursts). This cannot be achieved with only spike-generating currents but can be realized with two additional voltage-dependent current terms. There is a slow depolarizing current which reflects a slowly activating, persistent sodium current $I_{Na,p_{TH}}$ and a slow repolarizing current which is modeled as a calcium-dependent potassium

current $I_{K,Ca_{TH}}$. These currents activate below the threshold for spike generation, and their interaction can give rise to slow subthreshold oscillations (described in details in Braun et al., 2003b).

There are also diverse terms of synaptic currents. The term $I_{gj_{TH}}$ accounts for the gap junction currents between the two neurons. Additionally, both thalamic neurons are receiving synaptic input from the hypocretin neuron which introduces two more synaptic current terms: one for the transmitter glutamate $I_{glu_{TH}}$ and another one for the co-transmitter hypocretin $I_{hcrt_{TH}}$.

An external current $I_{ext_{TH}}$ is used to adjust the base-line activity of the thalamic neurons. A noise term $I_{noise_{TH}}$ is required to demonstrate the transitions between asynchronous and synchronized states.

4.2 Membrane currents

4.2.1 Leak currents

The leak currents of all neurons are modeled in the same way:

$$I_{l_i} = g_{l_i}(V_i - V_{l_i}), \tag{4.4}$$

with identical values for the leak conductances g_{l_i} and the equilibrium potentials V_{l_i}, where i stands for HCRT, GLU and TH.

4.2.2 Voltage-gated currents

All voltage-gated currents are implemented according to the general form as described in the Methods chapter. This leads to the following currents equations:

a) for the spike-generating currents appearing in all neurons (i = HCRT, GLU, TH):

$$I_{Na_i} = g_{Na_i} a_{Na_i}(V_i - V_{Na}), \tag{4.5}$$

$$I_{K_i} = g_{K_i} a_{K_i}(V_i - V_K), \tag{4.6}$$

b) for the additional subthreshold currents of thalamic neurons:

$$I_{Na,p_{TH}} = g_{Na,p_{TH}} a_{Na,p_{TH}} (V_{TH} - V_{Na}), \tag{4.7}$$

$$I_{K,Ca_{TH}} = g_{K,Ca_{TH}} a_{K,Ca_{TH}} (V_{TH} - V_K), \tag{4.8}$$

where V_{Na} and V_K are the equilibrium, i.e. the reversal, potentials of the fast and slow sodium and potassium currents.

The parameters g_{Na}, g_K, $g_{Na,p}$ and $g_{K,Ca}$ are the maximum conductances which can be achieved when all ion channels are activated through which the specific currents can flow. The maximum conductances for the spike-generating currents are much bigger then for the subthreshold currents to account for the strong voltage deflections during an action potential compared to smaller subthreshold oscillations.

In all cases, the **activation variables** a describe the actual ratio of activated (open) ion channels (normalized to 1) which changes as a function of voltage and time.

The voltage dependencies of the activation variables are provided by sigmoid curves (for details see Methods):

$$a_{Na_i\infty} = \frac{1}{1 + exp(-s_{Na}(V_i - V_{0_{Na}}))}, \tag{4.9}$$

$$a_{K_i\infty} = \frac{1}{1 + exp(-s_K(V_i - V_{0_K}))}, \tag{4.10}$$

$$a_{Na,p_{TH}\infty} = \frac{1}{1 + exp(-s_{Na,p}(V_{TH} - V_{0_{Na,p}}))}. \tag{4.11}$$

Such sigmoid curves are fully determined by their slopes s and half-activation potentials V_0. These values are completely identical for both of the spike generating sodium and potassium currents and are the same for all neurons. Only the slope for the slow sodium current is smaller and the curve is shifted to more negative values to achieve subthreshold activation (see Table 4.1). As an excep-

tion, the slow repolarizing current does not have a voltage-dependent activation variable. Its activation is directly connected to the slow depolarizing current (see below).

The time dependencies of the activation variables are bringing in the functionally most important dynamics. For simplicity, the fast spike-generating sodium current is considered to activate instantaneously, i.e. without any time delay. This can be justified, considering that it is in any case much faster than all the other currents.

$$a_{Na_i} = a_{Na_i\infty}. \tag{4.12}$$

It is the delayed activation of the fast potassium current which allows the even faster sodium current to produce the strong upward deflection of the action potential before the potassium current repolarises the membrane.

$$\frac{da_{K_i}}{dt} = \frac{a_{K_i\infty} - a_{K_i}}{\tau_K}. \tag{4.13}$$

The time delays of the slow subthreshold system of thalamic neurons are much longer (see Table 4.1). The depolarizing, subthreshold sodium current, similar to the fast potassium current, activates with first order time delay:

$$\frac{da_{Na,p_{TH}}}{dt} = \frac{a_{Na,p_{TH}\infty} - a_{Na,p_{TH}}}{\tau_{Na,p}}. \tag{4.14}$$

The equation for the slow repolarizing current has a different form. It is modeled as a simplified version of calcium-dependent potassium current:

$$\frac{da_{K,Ca_{TH}}}{dt} = \frac{-\eta I_{Na,p_{TH}} - k a_{K,Ca_{TH}}}{\tau_{K,Ca}}. \tag{4.15}$$

The factor η directly connects $I_{K,Ca}$ to $I_{Na,p}$. This can be justified because the $I_{Na,p}$-induced depolarization is the source for the inflow of Ca^{2+} ions through low-voltage activated calcium channels with subsequent opening of calcium-dependent potassium channels (for details see Braun et al., 2003a). The relaxation factor k physiologically reflects the elimination of intracellular calcium by calcium pumps. The values of η and k (see Table 4.1) in relation to the common time constant $\tau_{K,Ca}$ determine the time delays of activation and inactivation.

4.2.3 Synaptic currents

Synaptic currents are the major determinants for the system's behavior. They are either flowing through gap junctions between directly neighbored neurons or through transmitter-gated ion channels of chemical synapses.

Gap junctions are implemented for the coupling between the two thalamic neurons. They allow current flow in both directions

$$I_{gj_{TH}} = g_{gj_{TH}}(V_{TH} - V_{TH_{neighbor}}). \tag{4.16}$$

The gap junction current, again, is determined by the conductance and the driving force. $g_{gj_{TH}}$ reflects the compound conductivity which is made up by all the gap junctions between the two neurons. The conductivity is considered to be constant, and the gap junctions are assumed to be unspecific allowing all kind of ions to pass according to their driving force. Accordingly, the effective driving force for the gap junction current of an individual thalamic neuron is given by the difference between its own voltage and that of its neighbor. With only two neurons, the gap junction conductance is identical for both neurons, and the current for one neuron is the same as for the other but with opposite sign.

Chemical synapses are making the reciprocal connections between the hypothalamic glutamate and hypocretin neurons and also make the contacts from the hypocretin neuron to the thalamic neurons (see Fig.4.1).

Information transmission through chemical synapses is unidirectional and much more complicated than via gap junctions. It is initiated by presynaptic action potentials and goes through different steps until postsynaptic currents occur. The translation of the physiological processes into mathematical equations in a simplified, but functionally justified, form has been described in the Methods chapter. In the following I will emphasize on specific features of the different connections and their transmitter-gated currents.

The simplest connection is made by the projection from the glutamate interneuron to the hypocretin neuron which is mediated by the transmitter glutamate. According to the general form, the current equation can be written as:

$$I_{glu_{HCRT}} = g_{glu_{HCRT}} a_{glu_{HCRT}} (V_{HCRT} - V_{syn}). \tag{4.17}$$

Synaptic projections from the hypocretin neuron additionally make use of the

co-transmitter hypocretin which introduces a second current term at each synapse.

$$I_{glu_j} = g_{glu_j} a_{glu_j} (V_j - V_{syn}), \tag{4.18}$$

$$I_{hcrt_j} = g_{hcrt_j} a_{hcrt_j} (V_j - V_{syn}). \tag{4.19}$$

I_{glu_j} represents the glutamate part of the synaptic transmission and I_{hcrt_j} accounts for hypocretin-induced currents, whereby j stands for the currents at the glutamate (GLU) and the two thalamic (TH) neurons.

These equations for all the synaptic currents are formally identical to those of voltage-gated currents. The actual postsynaptic conductance is again calculated as the product of the maximum conductances g_{glu} and g_{hcrt} and the corresponding activation variables a_{glu} and a_{hcrt}. The driving force is given by the difference between the actual membrane voltage of the postsynaptic neuron V_j and the corresponding equilibrium (reversal) potential of the synaptic currents V_{syn}.

For simplicity, all synaptic equilibrium potentials are set to V_{Na} (+50mV). This value cannot be exceeded by the membrane voltage. Therefore, the driving force and, accordingly, the synaptic current are always negative, what leads to a depolarizing inward current, and the generation of excitatory postsynaptic potentials (EPSPs). Inhibitory postsynaptic potentials (IPSPs) can be modeled in a similar way but are not required for the present study.

Functionally important properties, again, are introduced by the voltage and time dependencies of the activation variables which can be modeled with formally identical equations as for the voltage-gated currents.

The synaptic activation variables change with first-order time delays

$$\frac{da_{glu_{HCRT}}}{dt} = \frac{a_{GLU_{rel}} - a_{glu_{HCRT}}}{\tau_{glu}}, \tag{4.20}$$

$$\frac{da_{glu_j}}{dt} = \frac{a_{HCRT_{rel}} - a_{glu_j}}{\tau_{glu}}, \tag{4.21}$$

$$\frac{da_{hcrt_j}}{dt} = \frac{a_{HCRT_{rel}} M - a_{hcrt_j}}{\tau_{hcrt}}, \tag{4.22}$$

and sigmoid voltage dependencies

$$a_{GLU_{rel}} = \frac{1}{1 + exp(-s_{syn}(V_{GLU} - V_{spike}))}, \tag{4.23}$$

$$a_{HCRT_{rel}} = \frac{1}{1 + exp(-s_{syn}(V_{HCRT} - V_{spike}))}. \tag{4.24}$$

Despite of the, principally, identical mathematical expressions, the physiological meaning of the synaptic variables and parameters is different from those of voltage-gated currents in major respects. These equations comprise several steps of synaptic transmission, from presynaptic transmitter release to the opening of transmitter-gated ion channels at the postsynaptic membrane (see Methods).

Most importantly, compared to voltage-gated channels, the activation of transmitter-gated postsynaptic channels does not depend on the postsynaptic membrane potential but on the transmitter concentration. Accordingly, the time dependencies (Eqs. 4.20-4.22) first of all reflect the accumulation and degradation/diffusion of the neurotransmitters in the synaptic cleft.

The accumulation is represented by $a_{GLU_{rel}}$ and $a_{HCRT_{rel}}$ and the elimination is proportional to actual activation values a_{glu} and a_{hcrt} which change with time constants τ_{glu} and τ_{hcrt}, respectively. The function of the additional multiplication factor M in the Eq.4.22 is described further below.

In case of glutamatergic transmission, which is considered to act via directly-gated ionotropic receptors (Fig. 4.2A,a), it can be expected, in a rough approximation, that the activation of postsynaptic receptors a_{glu} is directly proportional to the transmitter concentration.

The situation is different for the co-transmitter hypocretin which binds to metabotropic receptors. In this case, the activation of ion channels a_{hcrt} is mediated via a G-protein coupled second-messenger cascade (Fig. 4.2A,b) which introduces significant time delays. This is considered, in a simplified form, by a longer time constant τ_{hcrt} (see Table 4.1) which leads to physiologically appropriate time delays of the postsynaptic potentials (Fig. 4.2B).

The amplitude of the metabotropic hypocretin-induced current, and, therefore, its postsynaptic potential, is much smaller comparing to the directly-gated, ionotropic glutamate-induced currents. With the above equations, this physiolog-

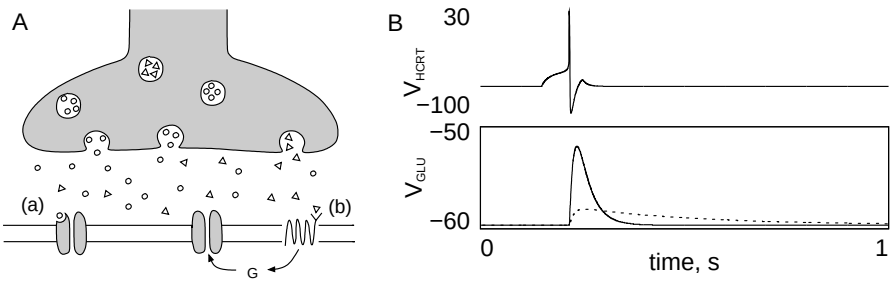

Figure 4.2: A: Simplified scheme of a synapse with ionotropic and metabotropic receptors. The presynaptic terminal contains vesicles with glutamate (circles) and hypocretin (triangles). Glutamate acts via fast, ionotropic receptors which are an integral part of the ion channels (a). Hypocretin binds to metabotropic receptors (b) which activate remote ion channels via a G-protein coupled second messenger cascade. B: Comparison of postsynaptic potentials from ionotropic and metabotropic receptors. A single presynaptic action potential in the hypocretin neuron (upper panel) induces postsynaptic potentials in the postsynaptic glutamate neuron (lower panel) of different amplitudes and time delays depending on the activation of ionotropic (solid line) or metabotropic receptors (dashed line).

ically well known effect must not be introduced separately but is a direct consequence of the longer time constants. The reason is, that the activation variable reaches smaller values during the short rise time which is given by $a_{GLU_{rel}}$ and $a_{HCRT_{rel}}$.

The parameters $a_{GLU_{rel}}$ and $a_{HCRT_{rel}}$ reflect transmitter release due the occurrence of presynaptic action potentials. This is considered by the steep sigmoid, almost threshold-like activation curves (Eqs.4.23 and 4.24). In these equations V_{GLU} and V_{HCRT} are the membrane potentials of the presynaptic cells. V_{spike} is the half-activation potential for transmitter release and s_{syn} is the slope of the curves, which is set to a high value to provide an almost step-like increase. V_{spike} and s_{syn} are set to determine the "threshold" of spike-dependent transmitter release and are identical for all neurons. Moreover only one sigmoid curve (Eq.4.24) is required for both glutamate and hypocretin which are released from the same presynaptic terminal of the hypocretin neuron.

4.2.4 Synaptic plasticity

The most important dynamics of homeostatic sleep-wake regulation are introduced by state-dependent changes of the efficacy of synaptic hypocretin effects. This is

implemented via the modulation function M that scales the activation variable of hypocretin-induced postsynaptic current (Eq.4.22). The idea is that (1) the synaptic efficacy of the neuropeptide hypocretin decreases with each spike of the hypocretin neuron and (2) this effect is antagonized by a continuous recovery process (see Chapter 2). Accordingly, the modulation function M comprises two terms, one for the recovery of the synaptic efficacy and one for its decrease:

$$\frac{dM}{dt} = \frac{M_{max}-M}{\tau_{inc}} - \frac{a_{HCRT_{rel}}M}{\tau_{dec}}. \tag{4.25}$$

The term for elimination is the second one which goes in with a negative sign. It depends on ($a_{HCRT_{rel}}$) which stands for the release of hypocretin into the synaptic cleft whenever the hypocretin neuron spikes. The degree of decrease with each spike is scaled by the time constant τ_{dec}. It additionally depends on the actual value of the modulation variable M which shall consider that the decline is smaller when the synaptic efficacy is already weakened. This can account for a reduced availability of hypocretin in the presynaptic terminal due to its spike-dependent release as well as for postsynaptic internalization of hypocretin receptors due to the activity-dependent hypocretin concentrations in the synaptic cleft.

The first term accounts for the recovery process, i.e. the increasing synaptic efficacy which may reflect, according to the above mentioned alternatives, a continuous supply of hypocretin by the neurons' and its transportation to the synaptic terminals or the re-embedding of postsynaptic receptors. Such a recovery process, of course, cannot continue to infinity but will go into saturation. Here, the maximum value is given by M_{max} which the recovery process approaches exponentially with a time constant τ_{inc}.

It is important to note that the recovery and depletion terms are not independent but interconnected via the common variable M. Although the spike-dependent degradation term does not play any role during the silent states of the hypocretin neuron, the recovery term is continuously active, also in the firing states where both functions superimpose.

4.2.5 External currents

External currents are introduced to account in a simplified form for additional inputs from other brain nuclei. These currents can be considered as unspecific,

compound synaptic input from a manifold of neurons. Such simplification is often used in neuronal network simulations, e.g. in the well known Hopfield networks (Hopfield et al., 1983; Gu and Liljenström, 2007).

In case of thalamic neurons, the external input goes to both neurons and is used to tune the activity pattern according to a physiologically reasonable dynamic state. In the model of the homeostatic network, only the hypocretin neuron receives external input. It is mostly used to activate the network with a simple current pulse which allows more clear illustration of the internal homeostatic dynamics without any superposition with continuous external modulation. Only for the demonstration of periodic sleep-wake transitions, the homeostatic network receives gradually changing input from the circadian pacemaker. It is implemented in the form of skewed sine function as proposed by for the original two-process model by Daan and colleagues (1984).

$$I_{circadian} = A_c(0.97sin(w_c t) + 0.22sin(2w_c t) + 0.07sin(3w_c t) \\ +0.03sin(4w_c t) + 0.001sin(5w_c t) + 1). \qquad (4.26)$$

The parameter A_c scales the amplitude and w_c is the angular frequency of the circadian current.

4.3 Stochastic components (noise)

Membrane currents, apart from their systematic modulation, always exhibit randomly appearing fluctuations. To account for such stochastic components, generally a separate term of a noise current is added. It is mostly implemented as Gaussian white noise which, according to Fox et al., (1988), is given by the equation:

$$I_{noise_{TH}} = \sqrt{(-4D/\Delta t)ln(a)}cos2\pi b, \qquad (4.27)$$

where Δt is the time step of integration, and $a, b \in [0, 1]$ are uniformly distributed random numbers. The noise intensity is adjusted by the parameter D which corresponds to the standard deviation of the Gaussian distribution of noise values.

Noise can make the simulations appearing physiologically more realistic while it does not necessarily introduce systematic changes of the model's dynamics. This is the case for the here described phenomena of the homeostatic sleep-wake

regulation. Therefore, the underlying principles are better illustrated without disturbances by noise.

A noise term only appears in the membrane equations of thalamic neurons. In this network of only two identical neurons with identical synaptic inputs, the noise current is needed for de-synchronization and, in a simplified form, can introduce typical effects of current fluctuations which naturally arise in larger networks (Postnova et al., in print a).

The full set of neuronal and synaptic equations constitutes a model system which allows to examine the dynamics of a homeostatic process and its interaction with a circadian signal and thalamic neurons on a physiological basis. The list of numerical parameters values is given in the Table 4.1.

For practical reasons, most of the simulations are run for seconds instead of hours. Only the simulation with the circadian current input which illustrates periodic sleep-wake transitions is run during "real-time" cycle of 24 hours. To achieve the appropriate synaptic changes within seconds, instead of hours, the time delays τ_{inc} and τ_{dec} of the function M have been adjusted by the factor of 3600 (seconds per hour). All other parameters are identical.

A: Voltage−gated currents

parameter / neuron	g_l	g_{Na}	g_K	$g_{Na,p}$	$g_{K,Ca}$	V_l	V_{Na}	V_K	$V_{0_{Na}}=V_{0_K}$	$V_{0_{Na,p}}$	$s_{Na}=s_K$	$s_{Na,p}$	τ_K
TH	0.1	1.3	1.75	0.22	0.35	−60	50	−90	−25	−40	0.25	0.09	3.5
HCRT	0.1	3.0	4.0	–	–	−60	50	−90	−25	–	0.25	–	2
GLU	0.1	3.0	4.0	–	–	−60	50	−90	−25	–	0.25	–	2

parameter / neuron	$\tau_{Na,p}$	$\tau_{K,Ca}$	η	k
TH	17	35	0.012	0.17

B: Postsynaptic and external currents

V_{syn}	V_{spike}	s_{syn}	τ_{glu}	τ_{hcrt}	τ_{dec}	τ_{inc}	τ_{dec} (Fig.5.8)	τ_{inc} (Fig.5.8)	M_{max}	A_c	ω_c	$A_{I\,ext\,HCRT}$
50	−20	1	30	300	920	7500	3312000	27000000	1	1	$2\pi/24$ [Hrs]	0.9

Table 4.1: Numeric values of the model parameters. A: The parameters of the individual neurons. Here TH, HCRT and GLU correspond to the thalamic, hypocretin and glutamate neurons, respectively. g_i are the maximum conductances for the ionic currents, and V_i are the equilibrium potentials. V_{0i} and s_i are the half-activation potentials and slopes for the voltage dependencies of the activation variables. τ_i are the time delays for the activation of the corresponding ionic currents. The factors η and k determine the dynamics of the Ca-dependent K current (for details see text). B: The parameters for the synaptic currents and external inputs. Here V_{syn} is an equilibrium potential for the transmitter-gated synaptic currents. V_{spike} and s_{syn} are the half-activation potential and slope, which are set to determine the threshold of the presynaptic spike-generation. τ_{glu} and τ_{hcrt} are the time delays for the activation of the postsynaptic currents due to the activation of the glutamate and hypocretin receptors respectively. τ_{inc} and τ_{dec} are the time delays of the modulation function M, and M_{max} is a maximum value of M. A_c and w_c are the amplitude and angular frequency of the circadian current, while $I_{ext_{HCRT}}$ is an amplitude of the current pulses applied to the hypocretin neuron. All of the conductances are given in $\mu S/cm^2$; voltages - in mV; half-activation slopes are in mV^{-1}; time delays - in ms; current amplitudes in $\mu A/cm^2$, and M_{mas}, η and k are dimensionless.

Chapter 5

RESULTS

The results section comprises three parts across which the model is continuously extended. It starts with simulations of homeostatic mechanisms (5.1) which then will be connected to a circadian process (5.2) and further to a model of thalamic synchronization (5.3).

The results demonstrated in the first two parts will be published in the December 2009 issue of Journal of Biological Rhythms (Postnova et al., in pint b). The results shown in the third part of the section are partly published and partly in pint in Journal of Biological Physics (Postnova et al., 2007b), Journal of Physiology Paris (Postnova et al., in print a) and Journal of Cognitive Neurodynamics (Postnova et al., in print c).

The first part about homeostatic sleep-wake regulation (5.1) describes how the proposed physiological mechanisms are translated into a mathematical model. This shall be done step by step to elucidate the dynamics which are introduced by the specific model components. The focus will be laid on the synapses with classical transmitter glutamate and activity-dependent co-transmitter hypocretin and on the excitatory feedback loop between the hypocretin and glutamate neurons. The dynamics of the homeostatic system shall further be illustrated in response to external disturbances which, for example, can reflect the effects of sleep deprivation and/or forced awakening by an alarm clock.

In the second part (5.2) the model of homeostatic mechanisms will be connected to a circadian input for the simulation of regular sleep-wake cycles on a real 24 hours time scale. Major emphasis will be laid on the elucidation of particular aspects of sleep-wake transitions which develop from the interactions of the

homeostatic and circadian processes at the level of neuronal impulse generation.

The third part (5.3), introduces a mathematical model of thalamic neurons with specific features for the generation of impulse groups (bursts) as well as of single spike activity. These neurons will be fed by the synaptic input from the hypocretin neuron of the homeostatic model with circadian input. It will be demonstrated that under influence of the introduced sleep-regulating processes the thalamic neurons can undergo the transitions between the asynchronous tonic firing activity and synchronized burst discharges according to the transitions between wakefulness and sleep.

Accordingly, as the outcome of these simulation studies, a simplified but physiologically plausible model of sleep-wake cycles can be presented. It connects homeostatic sleep mechanisms with circadian process and thalamic neurons and accounts for experimentally observed alterations of the activity pattern of hypothalamic wake-active neurons and for the associated changes of thalamic impulse patterns and synchronization states.

5.1 Homeostatic mechanism

The homeostatic regulation of sleep-wake cycles is ascribed to activity-dependent alterations of the synaptic efficacy of hypocretin neurons in the lateral hypothalamus (LHA). These neurons are reciprocally connected to local glutamate interneurons (Li et al., 2002). To emphasize on the dynamically relevant alterations of synaptic transmission, the model considers only two neurons (schematically illustrated in Fig.5.1). One represents the hypocretin neurons (HCRT) and synaptically innervates the other one, representing the glutamate neurons (GLU), via the co-release of glutamate (glu) and hypocretin (hcrt). The backward synaptic connection from the glutamate to the hypocretin neuron is modeled in a more simple way, i.e. with the release of only one transmitter - glutamate (glu).

The model parameters have been adjusted in such way that both neurons are silent without an external stimulus. Activation of the neuronal circuit is achieved with external stimuli which are exclusively applied to the hypocretin neuron. In this section, for an easier illustration of the internal homeostatic dynamics, these stimuli are represented by simple current pulses. The current amplitude is set to a level which allows (1) to induce firing of sufficiently high frequency in the

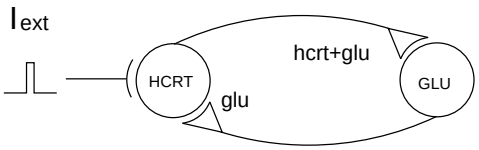

Figure 5.1: Schematic representation of the feedback model. The hypocretin (HCRT) neuron is synaptically innervating the glutamate (GLU) neuron with release of glutamate (glu) and co-release of hypocretin (hcrt). The synaptic connection from the glutamate neuron to the hypocretin neuron is solely made via glutamate release. The hypocretin neuron additionally receives external input in form of current pulses.

hypocretin neuron (2) to activate the glutamate neuron via synaptic transmission from hypocretin neuron and, thereby, (3) to keep the network in an active state through the excitatory reciprocal connections. The important point is that spiking in the glutamate neuron can only be induced by combined action of the transmitter glutamate and its co-transmitter hypocretin and that the synaptic efficacy of hypocretin can significantly change.

5.1.1 The glutamatergic synapse with co-release of hypocretin

The basic characteristics of the glutamatergic (glu) synapse with co-release of hypocretin (hcrt) can best be illustrated when the presynaptic firing is kept constant and alterations of synaptic efficacy can be neglected. This can be achieved when the reciprocal connection from the glutamate to the hypocretin neuron is eliminated ($g_{glu_{HCRT}} = 0$ in Eq.4.19) and the modulation function M of hypocretin transmission is kept constant at its initial value $M = M_{max}$, i.e. $dM/dt = 0$. An external current pulse is applied to induce a constant firing rate in the hypocretin neuron. The postsynaptic effects of the individual transmitters and their superposition are illustrated in Figure 5.2 which demonstrates the dynamically relevant feature of this synapse: with the chosen parameter settings neither glutamate nor hypocretin alone is sufficient for postsynaptic spike generation. It needs a combined action of both.

Glutamate acts via ionotropic receptors inducing fast postsynaptic potentials (PSPs) of comparably high amplitudes but with only minor superposition (c). The

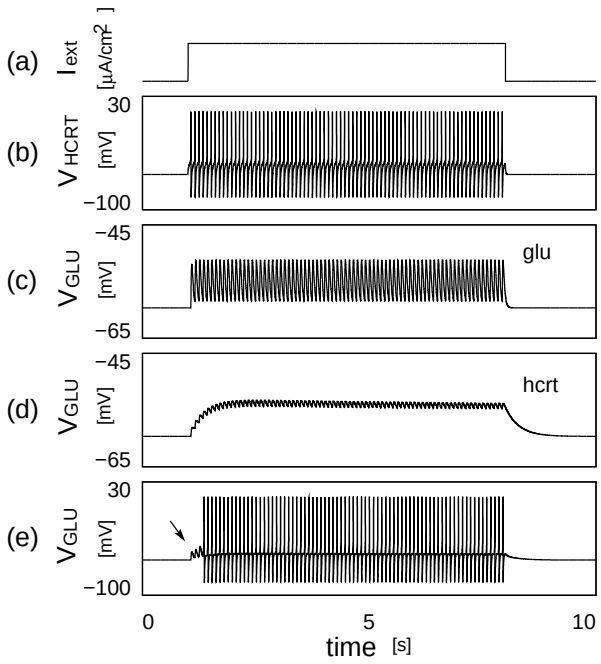

Figure 5.2: Postsynaptic potentials via ionotropic and/or metabotropic receptors in response to a presynaptic spike train. A depolarizing current I_{ext} (a) induces a continuous train of action potentials in the hypocretin neuron (b) which can initiate postsynaptic potentials in the glutamate neuron of different form depending on the postsynaptic receptors. Activation of glutamate receptors induces fast potential deflections (c), while postsynaptic potentials via hypocretin receptors superimpose to an almost gradual depolarization. The threshold for postsynaptic spike generation is only reached when the effects of both types of receptors are combined (e). The postsynaptic spikes appear with a certain delay ((e) arrow), because hcrt PSPs need time for sufficient superposition. The parameters' values are: (c) $g_{glu_{GLU}} = 0.15\mu S/cm^2$; (d) $g_{hcrt_{GLU}} = 0.2\mu S/cm^2$.

PSPs which are generated due to activation of metabotropic hypocretin receptors are smaller but have longer decay times and, therefore, can superimpose to reach an almost constant depolarization (d). Postsynaptic spikes can only be initiated when both effects are combined, i.e. when the fast, glutamate-induced PSPs act at a postsynaptic membrane which is additionally depolarized by hypocretin (e). This leads to a certain time delay between firing onsets of the pre- and postsynaptic neurons ((d) arrow) because it needs several presynaptic action potentials for sufficient summing-up of the hypocretin-induced PSPs at the glutamate neuron.

It is assumed that the relevant changes which can account for a homeostatic process appear at such glutamatergic synapses with co-release of hypocretin.

5.1.2 Activity-dependent change of hypocretin effects

In this neuronal model the homeostatic changes are attributed to alterations of the hypocretin synaptic efficacy which decreases during wakefulness and recovers during sleep. The decrease is related to the appearance of presynaptic spikes, while the recovery is a continuously ongoing process (for details see Model chapter).

To account for the state-dependent changes of synaptic efficacy, the modulation function M, which is scaling the activation variable of hypocretin transmission, has been introduced. According to the different mechanisms for decline and recovery, the function M has two different terms (Eq.4.25). Figure 5.3 illustrates how these mechanisms are acting together over a longer period of time. The feedback connection is still eliminated ($g_{glu_{HCRT}} = 0$ in Eq.4.19) to focus on the state-dependent alteration of the synaptic transmission from the hypocretin to the glutamate neuron.

In the example of Figure 5.3, a long-lasting depolarizing current I_{ext} (a) is applied to induce firing in the hypocretin neuron (b). Firing of the hypocretin neuron is of sufficiently high frequency to synaptically activate the glutamate neuron (c). The subsequent effects result from the activity-dependent synaptic plasticity. High frequency firing of the hypocretin neuron leads to the spike-dependent attenuation of the postsynaptic hypocretin effects (second term in Eq.4.25) which is much stronger than the recovery process (first term in Eq.4.25). This is reflected in the approximately exponential decrease of the modulation function M (d). As a consequence the synaptic efficacy is reduced, and the firing of the postsynaptic glutamate neuron slows down, and, finally, ceases when M goes below a certain

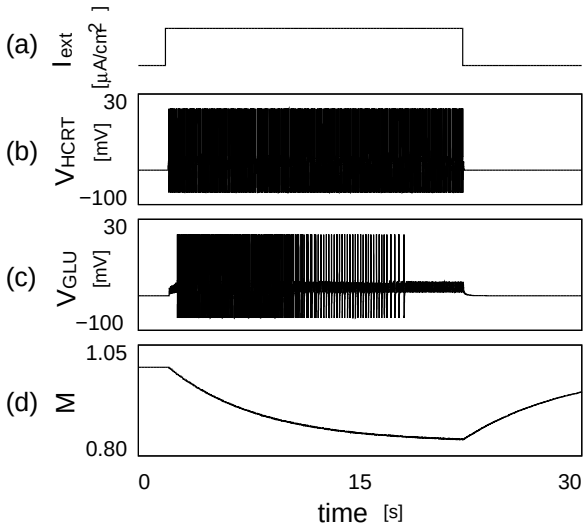

Figure 5.3: Effects of synaptic weakening on postsynaptic activity. A depolarizing current pulse I_{ext} (a) induces firing in the presynaptic hypocretin neuron (b) which synaptically activates the postsynaptic glutamate neuron (c). The postsynaptic firing rate depends on the value of M (d) which decreases with each presynaptic spike (spike-dependent decrease of the synaptic efficacy) and finally terminates postsynaptic firing. The M function can recover when also the presynaptic firing is switched off. The parameters' values are: $g_{glu_{GLU}} = 0.15\mu S/cm^2, g_{hcrt_{GLU}} = 0.135\mu S/cm^2$, $M_0 = 1$.

value (c).

The hypocretin neuron stops firing only when the stimulus is switched off. This allows the hypocretin synaptic strength to recover. The value of M increases towards its maximum M_{max} in an exponential form because the steepness of the recovery process is proportional to the distance between M and M_{max}.

Also the decline of M during the preceding firing phase becomes smaller the closer the M value comes to its minimum which is set to zero. However, it will never reach zero and also its time course is not precisely exponential. This is due to the fact that the recovery process continues also during the firing state. At high values of M the spike-dependent decline clearly predominates but it weakens along with decrease of M. Simultaneously, the recovery process is enhanced. After sufficiently long firing of the hypocretin neuron the value of M will reach a steady state, when the terms for decline and recovery become equally strong.

In this neuronal model, the terms for wake-dependent decrease and sleep-dependent recovery of the homeostatic function are interlinked. Both terms depend on the actual value of M (see Eq.4.27). In the firing state they co-exist because recovery is a continuously ongoing process. These interdependencies can lead to complex dynamics, especially, when the reciprocal connections are considered.

5.1.3 Effects of reciprocal excitatory connections

Completing the feedback loop by a reciprocal excitatory connection from the glutamate to the hypocretin neuron introduces new, important dynamics. This backward connection is realized via a simple glutamate synapse which is sufficiently strong to induce a postsynaptic action potential with each presynaptic spike.

A particularly prominent property of the reciprocal excitatory feedback loop can immediately be recognized in Figure 5.4. It illustrates that even a brief stimulus (a) can induce ongoing firing in both neurons (b). The stimulus has to be of sufficient strength and duration to generate a short train of spikes in the hypocretin neuron, which can activate the glutamate neuron. Once both neurons are firing, no further external input is required because spiking is sustained by the reciprocal connection.

Firing can persist as long as it is not interrupted by an external hyperpolarizing input or by an intrinsic process. In the presented model, it is an intrinsic process,

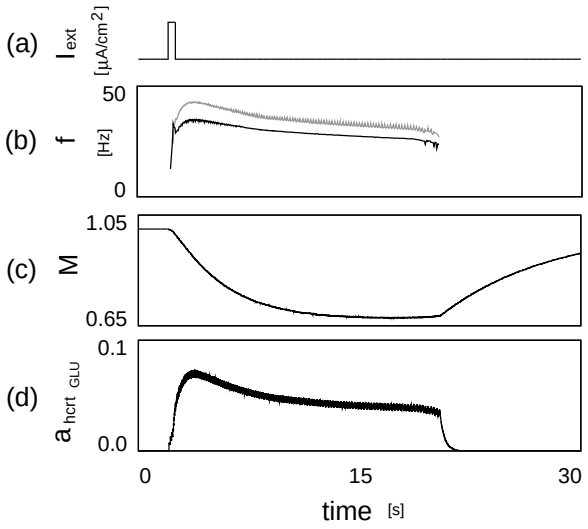

Figure 5.4: Activity of reciprocally coupled neurons during synaptic weakening. A short depolarizing stimulus (a) induces firing in the hypocretin neuron ((b) firing rate in black) which synaptically activates the glutamate neuron ((b) gray). Firing sustains after the stimulus due to the reciprocal excitation. It is switched off because of the activity-dependent decline of the modulation function M (c) which attenuates the activation variable $a_{hcrt_{GLU}}$ (d) of the hypocretin-induced postsynaptic current. The parameters' values are: $g_{glu_{HCRT}} = 0.196\mu S/cm^2$, $g_{glu_{GLU}} = 0.15\mu S/cm^2$ and $g_{hcrt_{GLU}} = 0.2\mu S/cm^2$, $M_0 = 1$.

i.e. the spike-dependent decline of the synaptic efficacy M (c). The decline of M leads to a decrease of the activation variable $a_{hcrt_{GLU}}$ (d). This decreases the firing rate of the glutamate neuron (gray line in (b)) which, in turn, weakens the glutamatergic input to the hypocretin neuron. Consequently, the firing rate in the hypocretin neuron slows down (black line in (b)).

The decreasing firing rate of the hypocretin neuron additionally weakens the hypocretin synaptic transmission due to the frequency-dependent superposition of postsynaptic potentials. This effect is reflected in the activation variable $a_{hcrt_{GLU}}$ which depends not only on the modulation function M but also on the variable a_{HCRT_∞} (see Eq.4.22). This variable is activated by the occurrence of presynaptic spikes and induces long-lasting postsynaptic effects according to the time constant τ_{hcrt}. This allows superposition of postsynaptic potentials depending on the intervals between the presynaptic spikes, i.e. the presynaptic firing rate.

The reduction of the activation variable $a_{hcrt_{GLU}}$ (d) due to the frequency decline is a functionally important property of this feedback loop. The decrease continues even when the modulation function M (c) slowly approaches a steady state and, finally, leads to the cessation of firing. At this point, the synaptic activation variable drops to zero, while the synaptic efficacy starts to gradually recover.

The transition from firing to silence in the neuronal model may be related to the transition from wakefulness to sleep on the behavioral level. In this model, it needs only a short external (wake-up) stimulus for the induction of initial firing. All subsequent changes occur due to the internal dynamics of the system: 1) The neurons keep firing (wakefulness) due to the reciprocal excitatory connections; 2) The transition to silence (sleep) occurs due to the decreasing efficacy of hypocretin transmission.

The increase of the modulation variable M in the silent (sleep) state can be taken as an indicator of decreasing sleep intensity in the course of night, and its decrease during the firing (wake) state may reflect an increasing sleep pressure. The activation variable $a_{hcrt_{GLU}}$ can be associated with such a physiological parameter like alertness. It declines in the course of the active (wake) state and is zero during the silent (sleep) state. Especially, it depends not only on the homeostatic variable M but also on the firing rate. This means that alterations of the firing rate by any external stimuli can have direct impact on the synaptic transmission and, thereby, can overwhelm the internal homeostatic effects.

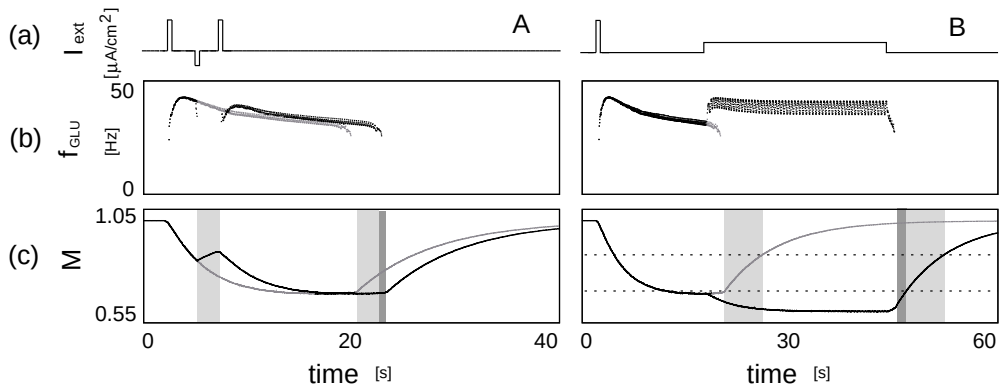

Figure 5.5: Interruption (A) and prolongation (B) of neuronal firing (awake state) by external stimuli. External currents (a) are applied to the hypocretin neuron. Panel (b) shows the frequency of the glutamate neuron and (c) the synaptic efficacy M. For comparison, gray curves show the responses under control conditions when only the first current pulse is applied (same as in Figure 5.4). Dashed boxes in (c) indicate the functionally relevant deviations. Time differences are emphasized by their dark parts. A: A transient interruption of firing (nap) results in a lengthening of the total firing state (awake time) B: Enforced prolongation of firing leads to a decline of M to lower values which lengthens the synaptic recovery. The parameters' values are the same as in Figure 5.4.

5.1.4 State-dependent effects of external stimuli

Functionally relevant alterations of the system's dynamics during the silent (sleep) or firing (wake) state can be assessed by the system's response to external stimuli when they are applied in different states. The simulations in Figures 5.5 and 5.6 illustrate well known phenomena which can be observed when regular sleep-wake cycles are disturbed, e.g. by a nap during the wake-phase (Fig.5.5A), by sleep deprivation (Fig.5.5B), or by wake-up stimuli during the night (Fig.5.6).

Figure 5.5A illustrates the effect of a transient interruption of the firing state (black curves, only the frequency of the glutamate neuron is shown) in comparison with an undisturbed state (gray curves) which already has been presented in Fig.5.4. In both cases, the neurons are activated by a short current pulse (a). Interruption of firing by a hyperpolarizing pulse allows the modulation function M ((c) black) to recover. When firing is reinstalled by a second depolarizing pulse, M has reached a much higher value than before the hyperpolarizing input or under control conditions. Accordingly, it needs more time to reduce the function M to

a value where firing can no longer be sustained. The transient interruption of 2 s (indicated by the light dashed bars) is even overcompensated by additional 0.7 s prolongation (dark bar) of the firing state.

The transient interruption of an otherwise ongoing firing may have its behavioral correlate with a nap and can demonstrate its positive effects. It does not only extend the waking time but also can enhance the alertness. The firing rates after the nap are slightly higher than under control conditions.

The example in Figure 5.5B is illustrated in the same way as in Figure 5.5A, showing the control curves from Figure 5.4 in gray. In this example an additional depolarizing current is applied (a) to increase the firing rate of the hypocretin and glutamate (b) neurons and, thereby, to prevent the transition to a silent state. This leads to a prolonged decrease of the modulation function M ((c), the lower dotted line indicates the level of M where transition to silence occurs under control conditions). When the external stimulus is turned off, the neurons switch to the silent state and the function M starts to recover. However, due to the lower starting point, a significantly longer time is required until M reaches the threshold value where firing could be reinstalled by the same current pulse as before ((c), upper dotted line). Compared to control conditions, the recovery time is 1.5 s longer (see light and dark gray bars in (c)).

In behavioral terms, this effect of the prolonged firing may reflect externally induced sleep deprivation. It results in a subsequent sleep rebound which is indicated by the lengthening of the recovery process.

The impact of synaptic recovery to the reactivation of the neurons is illustrated in Fig.5.6. In these simulations, excitatory stimuli of identical duration and amplitude have been applied at different times after the transition to silence. In Figure 5.6A, after only a short time of silence, the excitatory stimulus (a) induces spikes in the hypocretin neuron (b) which, however, cannot activate the glutamate neuron (c). The slightly recovered value of M (d) is too low to induce strong enough hypocretin effects (e). When the same stimulus is set later in the silent phase (Fig.5.6B), after further recovery of M, the glutamate neuron can be activated. However, firing stops when the stimulus is switched off. In this case, the firing frequency is still too low for sustained activation of the reciprocal excitatory circuit. When the stimulus is applied after a slightly longer recovery period (Fig.5.6C), sustained activation can be induced. At this point, M still has not reached its previous maximum value. This means that its lower threshold

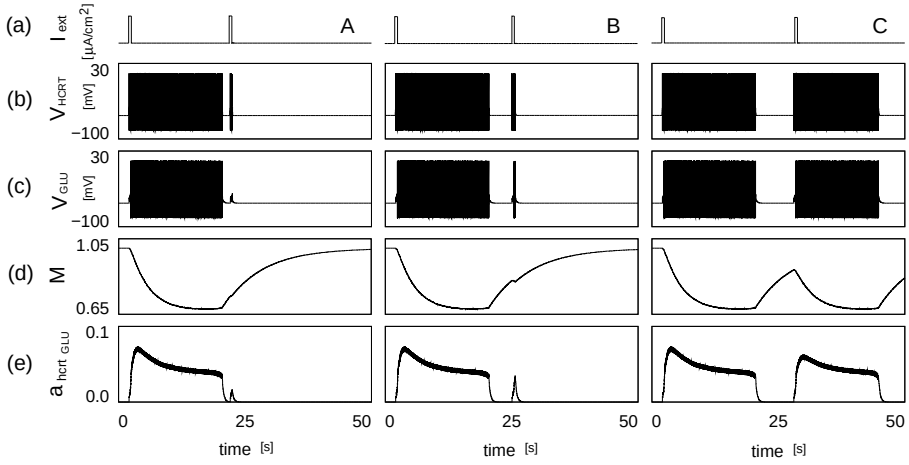

Figure 5.6: State-dependent effects of external stimuli during synaptic recovery (sleep). The first of the two current pulses (a) initiates long lasting firing in both the hypocretin (b) and glutamate (c) neurons. Firing is terminated after 20 seconds by the decreasing value of M (d) and the, thereby, attenuated hypocretin activation variable $a_{hcrt_{GLU}}$ (e). The second pulse is applied at different times after the cessation of firing: 22 s in A, 25 s in B and 28 s in C. These pulses are sufficiently strong to induce firing in the hypocretin neuron. However, early in the recovery state (A), the glutamate neuron cannot be activated. At a later state with stronger recovery (B), the glutamate neuron fires but stops when the stimulus is switched off. With a slightly longer delay (C), the synaptic efficacy has become strong enough for the induction of sustained firing. The parameters' values are the same as in Figure 5.4.

for spike-generation is reached in shorter time, and that the firing state is clearly shorter than before. This effect reflects a general principle, similar to the previous examples: the actual value of M at a moment of transition influences the time course of the following state.

Translated to the behavioral level, the shortening of the firing state after a shortened state of silence can be understood as falling asleep earlier after a short night. The different effects of external stimuli in different sleep phases may fit to the observation that a wake-up stimulus is more effective towards the morning. Of course, a sufficiently strong and long-lasting stimulus can bring the system in an active (wake) state at any time.

The following chapter shall demonstrate that these homeostatic mechanisms, connected to a circadian process, allow to simulate cyclic transitions between sleep and wake states in a physiologically appropriate time relation. It will also elucidate new, dynamically important properties which arise from the interaction of the two processes on a neuronal level.

5.2 Circadian drive

Until now the focus was laid on the functional characteristics of the proposed homeostatic process and, therefore, firing was initiated by external current pulses. In the following simulations a periodic, gradually changing current is applied, instead of the pulse-like stimuli, in order to simulate a circadian modulation of the hypocretin neurons by direct or indirect projection from the SCN (Fig. 5.7).

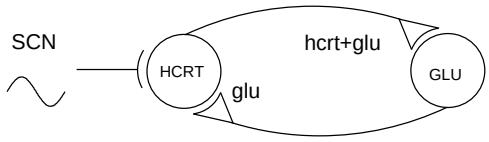

Figure 5.7: Schematic representation of the homeostatic model driven by a circadian current. The hypocretin (HCRT) neuron is synaptically innervating the glutamate (GLU) neuron with release of glutamate (glu) and co-release of hypocretin (hcrt). The synaptic connection from the glutamate neuron to the hypocretin neuron is solely made via glutamate release. The hypocretin neuron additionally receives external input from the circadian pacemaker in the SCN in a form of a skewed sine wave.

The shape of the circadian current is the same as in the two-process model (Daan et al., 1984). The amplitude of the circadian current was adjusted to the threshold of spike initiation so that the hypocretin neuron is activated a couple of hours before the circadian current reaches its maximum. This is related to the observation that the maximum activity of the SCN neurons is achieved during the subjective day, some hours after awakening (for review see Mistlberger, 2005).

To demonstrate that the homeostatic mechanisms, which so far have been simulated on a time scale of seconds, can easily be transferred to a realistic time scale of hours, the simulations in Figure 5.8 were run with 24 hours cycle periods. The adjustments have been made by multiplication of the decline and recovery parameters (τ_{dec} and τ_{inc}) of function M by 3600 (seconds per hour). This leads to a longer time of sustained firing and to a longer recovery phase, but does not change the principle model dynamics.

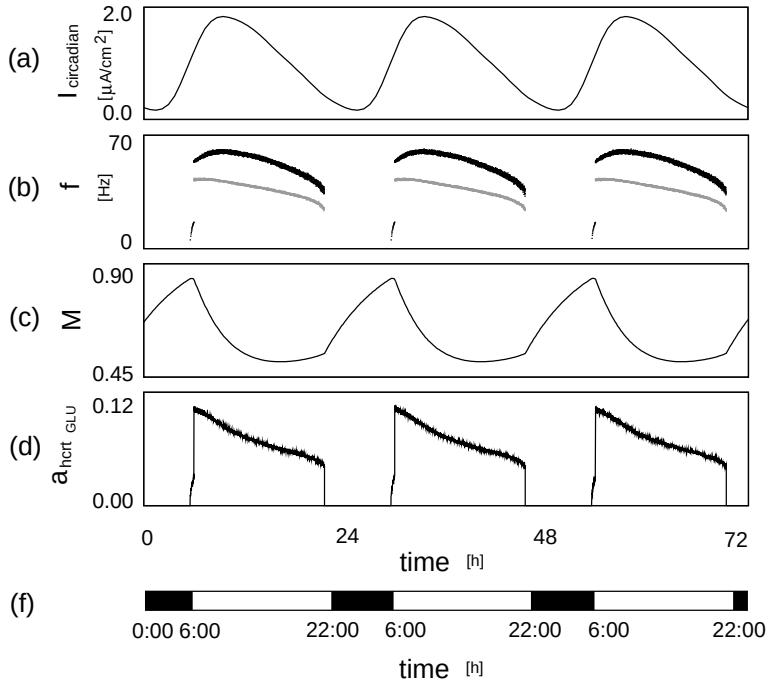

Figure 5.8: The influence of a circadian current on the homeostatic dynamics. An external current (a) is applied to the hypocretin neuron in form of a skewed sine-wave with a period of 24 hours. At a certain current value, the hcrt neuron starts to fire ((b) black curve) what, with a slight delay, induces spikes in the glutamate neuron ((b) gray curve). These transitions appear when also the modulation function M (c) has reached a comparably high value. During the active state, M decreases but already has turned to slightly increasing values when the transition to the silent state occurs. This transition appears due to the still decreasing activation variable $a_{hcrt_{GLU}}$ (d) which is essentially caused by the still decreasing current input (a). The parameters' values are: $g_{glu_{HCRT}} = 0.299\mu S/cm^2$, $g_{glu_{GLU}} = 0.1\mu S/cm^2$ and $g_{hcrt_{GLU}} = 0.13\mu S/cm^2$.

Additional slight adjustments, not related to the up scaling of the simulation time, were made with regard on the maximum conductances of postsynaptic currents (for the values see figure caption). These adjustments were required to compensate for the different forms of the input current. A pulse-like stimulus only transiently interferes with the model dynamics, while the circadian input is continuously modulating the dynamics of the homeostatic feedback system. This has several quantitative as well as qualitative consequences.

When the circadian input (a) exceeds the critical value of 0.82 $\mu A/cm^2$ it initiates firing in the hypocretin neuron ((b), black curve) which subsequently activates the glutamate neuron ((b), gray curve). In the first phase of the active state, the firing frequency of the hypocretin neuron is essentially determined by the strong circadian input which induces much higher firing rates than the short current pulse. This leads to an immediate and strong decline of the modulation function M (c) which also reduces the activation variable $a_{hcrt_{GLU}}$ (d). Consequently, the firing rate of the glutamate neuron also decreases ((b), gray curve) even as the firing rate of the hypocretin neuron ((b), black curve) still increases.

The initial strong decline brings the modulation function M to such low values that the ongoing recovery process begins to dominate. Towards the end of the active phase, M goes through a minimum and starts to increase. Nevertheless, the activation variable continues to decrease which, now, is the result of the decreasing firing rate. This frequency effect could also be seen in the previous examples. In the actual simulation it is additionally strengthened by the still decreasing input from the circadian pacemaker current. Finally, firing is terminated by the further decrease of the activation variable. The transition to silence happens at an intermediate state of sufficiently low values of the circadian input and the homeostatic function. After recovery firing is reinstalled by the circadian input which activates the hypocretin neuron. The glutamate neuron follows with a short delay.

Altogether, the homeostatic model with circadian input allows to simulate the major characteristics of hypocretin neurons, i.e. their transitions between silent and firing states in agreement with the experimental observations of the neuronal activity along the sleep wake-cycles. These simulations additionally have elucidated dynamically important interactions between the two sleep-regulating processes. Particularly, it has been shown that the transitions between sleep and wakefulness can arise from the intrinsic system's properties and do not require the

explicit sleep and wake threshold as introduced in the original two-process model (Daan et al., 1984). This is the result of the mechanism-based approach where the different sleep- and wake-related processes can interact on a physiological level of neuronal activity and synaptic transmission.

The following section shall demonstrate that this modeling concept can also be used to account for associated changes in other sleep-related brain nuclei, specifically, for the alterations of thalamic firing patterns and synchronization states as major markers of sleep-wake transitions.

5.3 Hypothalamic modulation of thalamic synchronization

The previous simulations have demonstrated the dynamics of the proposed homeostatic mechanism of sleep-wake regulation, including its interaction with the circadian process. In the present section the combined action of both homeostatic and circadian hypothalamic processes on the sleep-wake activity of thalamic neurons is examined (Fig.4.1).

Thalamic activity exhibits significant differences in sleep and wake states which are closely associated with accompanying changes in the EEG. There is unsynchronized, single-spike activity (tonic firing) during wakefulness which changes into synchronized discharges of impulse groups (bursting) at the transition to sleep (McCormick and Feeser, 1990). This is assumed to be one of the mechanisms by which the gate of sensory information transmission through thalamus to cortex is closing, and the brain goes from a state of conscious perception in to an unconscious sleep. It is easy to understand that a strongly coupled neuronal network with synchronized activity reacts less sensitive on a specific external stimulus then the network of neurons in different firing states.

Despite the undoubtedly high physiological relevance of these transitions, it is still under debate from what mechanisms they may originate. The computer simulations in the following sections shall demonstrate that the experimentally documented projections from hypocretin neurons in the lateral hypothalamus to thalamic neurons (Peyron et al., 1998; Sakurai et al., 2005) can play an important role in switching the thalamic neurons into different synchronization states according to the states of sleep and wakefulness.

The simulations, again, are performed with a minimal model which consists of only two gap junction coupled thalamic neurons. In this case, the functionally relevant properties are introduced by two additional, voltage-dependent ionic currents that allow the generation of broad variety of impulse patterns, including tonic firing and burst discharges (see Chapter 4). These two gap junction coupled neurons receive synaptic input from the hypocretin neuron of homeostatic model which is driven by a circadian input, as described in the previous section.

In the following section (5.3.1), first of all, the specific dynamical properties of the individual thalamic neurons will be briefly described with special regard to the

transitions from tonic firing to burst discharges. The second part (5.3.2) illustrates that the alterations of the firing pattern are sufficient to bring gap junction coupled neurons from an asynchronous to a synchronized state without the need to change the coupling strength. The third part (5.3.3), finally, connects these model neurons to the homeostatic model with circadian drive. It will demonstrate that the typical sleep-wake related alterations of impulse patterns and synchronization states in thalamic neurons can be introduced by synaptic input from the control centers in the hypothalamus.

5.3.1 Neuronal impulse pattern

The thalamic neurons are modeled in a form which allows them to develop slow membrane potential oscillations for the generation of grouped impulse sequences (bursts). This is achieved by the implementation of two so-called subthreshold currents. In comparison to the spike-generating currents, these are activated at lower membrane potentials (below the threshold of spike generation) and with much longer time delays (see model description).

The other functionally important feature is that with appropriate tuning of the model parameters or in response to external stimuli the same neuron can also exhibit tonic, single-spike activity. An example is shown in Figure 5.9 where an external, linearly increasing depolarizing current $I_{ext_{TH}}$ has been applied to smoothly tune the model neuron from an originally bursting into a tonic firing activity state.

The voltage traces of the short simulation in Fig.5.9A illustrate how the slow, burst-generating oscillations disappear leading to a pacemaker-like tonic firing activity. The bifurcation diagram of interspike intervals in Fig.5.9B comprises a much higher number of spikes and demonstrates that these transitions include a broad range of chaotic dynamics. At the beginning, the bifurcation diagram shows two lines of short and one line of longer intervals according the short intraburst intervals and longer burst-pauses. At the end of the simulation there is a single line of intervals corresponding to the regular single-spike discharges.

In the intermediate state, the interspike intervals of this completely deterministic simulation exhibit a strong variability which clearly indicates deterministic chaos. This happens via period-doubling bifurcations which means that single lines of intervals bifurcate into two lines which then bifurcate again and so forth

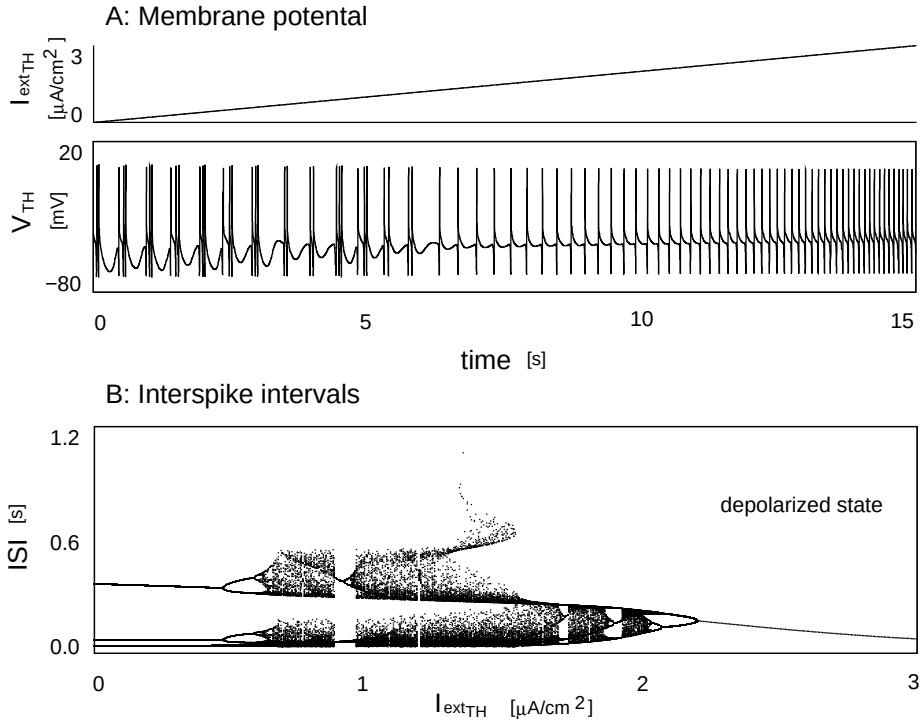

Figure 5.9: Bursting-to-tonic transition in the model of an individual thalamic neuron. A: Voltage traces (V_{TH}) of the model neuron in response to a depolarizing current input with linearly increasing amplitude from the $I_{ext_{TH}} = 0$ to $I_{ext_{TH}} = 3$ $\mu A/cm^2$ within 15 seconds. B: Bifurcation diagram of the interspike intervals (ISI) which is obtained with the corresponding but constant current values which have been increased in steps of 0.01 $\mu A/cm^2$) over the same interval of the applied current. It is obtained by recording of time intervals between subsequent spikes and demonstrates the stable states of a neuron at the corresponding value of the depolarizing input.

(Feigenbaum, 1978). This soon leads to an irregularly appearing mixture of interspike intervals. This is a classical way from regular activity to chaos (Strogatz, 1994). The chaotic regime additionally includes a homoclinic bifurcation which is indicated by the appearance of very long interspike intervals. It separates a burst-like chaotic regime, indicated by two separated bands of chaotically distributed intervals, from a regime of more uniformly distributed intervals in the neighborhood of the regular tonic firing regime.

Although the bifurcation structure of this model has been intensively examined and described in many details (e.g. Braun et al., 2000; Feudel et al., 2000) the underlying system dynamics are not yet fully understood. More importantly, there are clear indications of chaotic dynamics also in experimental data which, likewise, were preferably seen at the transitions from bursting to tonic discharges (Braun et al., 1999). This suggests that the model, although simplified, provides a physiologically important features, especially with regard to the functionally relevant impulse pattern alterations.

In this example the external depolarizing current can represent a compound synaptic input to the thalamic neurons which tunes them from bursting to tonic firing as it is the case at the transition from sleep to wake states.

5.3.2 Synchronization at tonic-to-bursting transitions

Transitions from tonic firing to bursting in thalamic neurons are closely correlated with the transitions from wakefulness to sleep which are going along with another functionally important phenomena - the transitions form asynchronous to synchronized activity. There are several concepts, partly also mathematical models (e.g. Hill and Tononi, 2005; Bazhenov et al., 2002), that tend to explain the sleep-wake related alteration of activity patterns and synchronization states.

This study focuses on the transitions between tonic firing and burst discharges of individual neurons which, in agreement with experimental data, can be achieved with a simple current injection (Wallenstein, 1994). The following simulation data shall illustrate that the changes of the activity patterns are sufficient to account for the alterations of synchronization states.

The map of synchronization states in Figure 5.10 is based on a detailed computational study with analysis of phase differences between the action potentials of two electrotonically coupled neurons. In this figure changes of synchronization

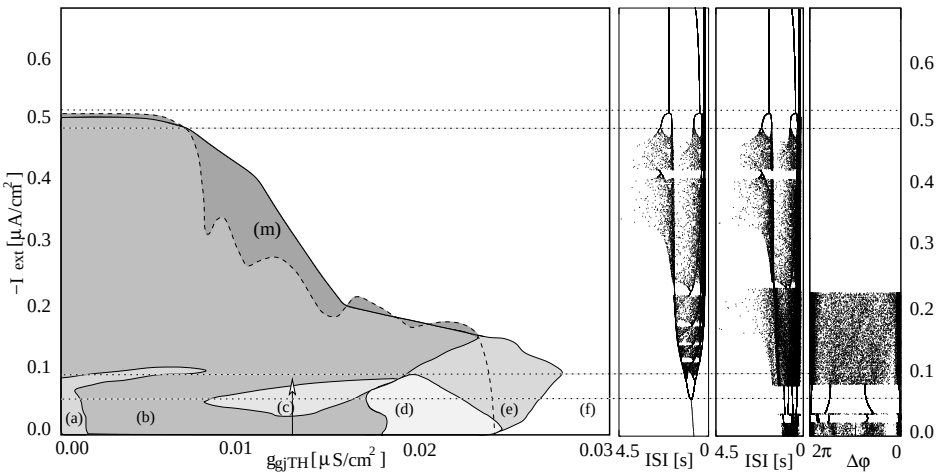

Figure 5.10: Map of synchronous states for two electrically coupled neurons at the tonic-to-bursting transitions on the plane of parameters: coupling strength ($g_{gj_{TH}}$ mS/cm^2) and external current (I_{ext} $\mu A/cm^2$). The panels on the right show (1) the bifurcation diagram of interspike intervals for the uncoupled as well as for in-phase synchronized neuron, (2) the bifurcation diagram of interspike intervals for the coupled neuron at the coupling $g_{gj_{TH}} = 0.013$, and (3) the bifurcation diagram of the phase difference between the spikes of two neurons at the coupling $g_{gj_{TH}} = 0.013$. The letters indicate areas of different synchronous states and asynchronous activity: (a) out-of-phase of period 1, (b) asynchronous, (c),(d) out-of-phase of higher period, (e) almost in-phase chaotic state, (f) in-phase synchronization. The area (m) denotes to a particular area of multistability of asynchronous behavior and in-phase synchronization. The dotted lines indicate the zones of transitions between spiking patterns of individual and in-phase synchronized neurons from tonic firing to chaos (lower lines) and from chaos to bursting (upper lines). For details see Postnova et al., 2007b.

activity are shown as a function of the coupling strength (abscissa) and current injection (ordinate). *This study has been performed with the same neurons as implemented here, although with slightly different parameter settings. Without external current these neurons are in a tonic firing state what, accordingly, means application of a hyperpolarizing current to tune them to burst discharges.* The different settings do not affect the physiologically relevant dynamics. The interdependencies between activity pattern and synchronization will briefly be summarized (for details see Postnova et al., 2007b; Postnova et al., in print a).

The map in Figure 5.10 shows a white area and several gray shadowed areas which indicate different synchronization states, according to their classification by the phase differences $\Delta\varphi$ between the action potentials of the two neurons. The white area represents in-phase synchronization while in all other areas non-zero phase differences between the spikes were recorded.

In deterministic simulations of two identical, gap junction coupled model neurons at different initial conditions in-phase synchronization is only a question of sufficiently high coupling strength (abscissa). The value of coupling strength for in-phase synchronization, however, strongly differs depending on the neurons activity pattern which is tuned by current injection (ordinate). The first panel to the right side from the map shows the bifurcation diagram of interspike intervals that would be obtained with an individual, uncoupled neuron. The second one shows the bifurcation diagram of one of the coupled neurons, and the third one shows the phase differences between the spike-times of the two coupled neurons. The last two diagrams are made at an intermediate, constant coupling strength ($g_{gj_{TH}} = 0.013\mu S/cm^2$, indicated by the upward arrow in the map). They demonstrate that transition to in-phase synchronization can be achieved simply by current injection (slightly above 0.2 $\mu A/cm^2$) due to associated alterations of the impulse pattern, noteworthy, in direction to burst discharges.

This example of bifurcation diagrams and phase-differences is drawn for one specific coupling strength. The map, additionally, illustrates that as soon as the two neurons exhibit regular bursts, this minimal model will go into in-phase synchronization at all, also infinitely small, coupling strengths. In the tonic firing regime much higher coupling strengths will be required for in-phase synchronization. This holds true also at the transitions from wakefulness to sleep.

5.3.3 Modulation by hypocretin

Based on the above described mechanisms suggesting neuronal synchronization as the result of tonic-to-bursting transitions, the model of thalamic neurons can now be connected to the hypothalamic model of homeostatic sleep-wake regulation.

In this model, no other external current to thalamic neurons than a synaptic input from the hypocretin neuron is considered. However, the hypocretin neuron can only provide external current during the active wake states. During the sleep states, this external excitation is missing. This means that in the sleep the thalamic neurons can generate burst discharges according to their intrinsic properties (see section 5.3.1). Burst discharges necessarily bring the gap junction coupled neurons into a synchronized state corresponding to thalamic activity pattern during sleep (see section 5.3.2).

To tune the thalamic neurons out of the bursting into the tonic firing mode, a depolarizing current input is required. Now it is provided by the synaptic input from the hypocretin neuron. However, once the two deterministic thalamic neurons are in-phase synchronized they will continue firing synchronously, irrespective of the actual impulse pattern. In-phase synchronized systems have to be brought out of the synchronized state by external perturbations. In physiological systems, which are never free of perturbations, this will happen naturally. In modeling studies, when no specific perturbations are implemented, such effects are usually introduced by stochastic components, i.e. a noise term (see Eqs.4.3 and 4.27). Therefore, while all the other simulations were run under completely deterministic conditions, the synchronization simulations were run with addition of noise which, in this case, is a functionally relevant component for de-synchronization.

The simulation results are illustrated in Figures 5.11 and 5.12. The calculations were again made with short cycle periods of 24 seconds (see explanation at the end of Chapter 3). As demonstrated before this does not make any difference with regard to the principle dynamics. The simulations can easily be transformed to 24 hours cycles according to the time bar in Fig.5.11(e).

Figure 5.11 shows the circadian input (a) and the, already known, transitions of the hypocretin neuron from silent to firing states (b). The corresponding activation variable of the hypocretin-induced synaptic currents is demonstrated in (c). Additionally, the voltage trace of one of the thalamic neurons is shown (d, the traces for both neurons look similar), which exhibit the changes of firing activity

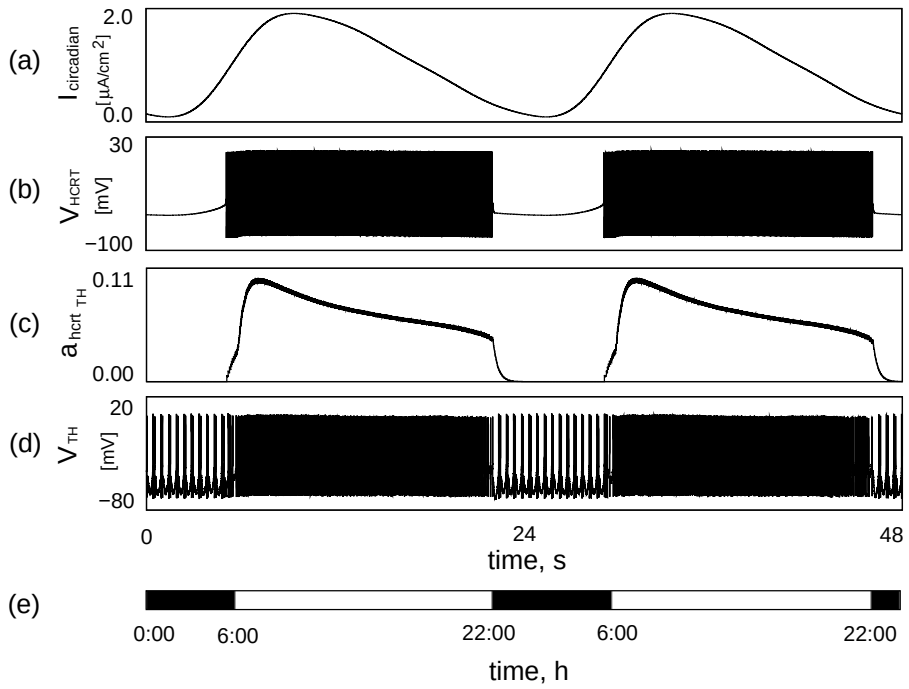

Figure 5.11: The sleep-wake activity of the thalamic neurons driven by the homeostatic and circadian mechanisms. An external circadian current (a) is applied to the hypocretin neuron in form of a skewed sine-wave with a period of 24 s which should reflect the daily 24 hours cycles (e). At a certain current value, the hypocretin neuron starts to fire (b) what induces tonic firing activity in the thalamic neurons (d). The transition to silence in the hypocretin neuron results from the decreasing activation variable $a_{hcrt_{TH}}$ (c) that is essentially caused by the decreasing current input (a). When the hypocretin neuron is silent the coupled thalamic neurons are switching to bursting discharges. The parameters for the hypocretin neuron are the same as in Fig.5.8, besides of the time delays of the modulation function M, which are divided on 3600 to simulate sleep-wake transitions with 24 seconds cycle. The parameters for the thalamic neurons are: $I_{ext_{TH}} = 0$, $g_{glu_{TH}} = 0.1\mu S/cm^2$ and $g_{hcrt_{TH}} = 0.13\mu S/cm^2$, $g_{gj_{TH}} = 0.01\mu S/cm^2$ and $D = 0.3$.

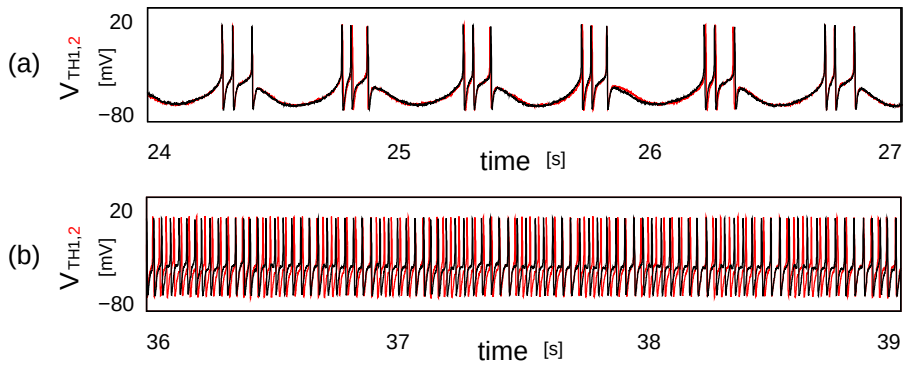

Figure 5.12: Synchronization states of the thalamic neurons during sleep-wake cycles. The membrane potentials of the electrically coupled thalamic neurons (two neurons are indicated by red and black colors) are in-phase synchronized in the burst discharges during sleep state (a) and are asynchronously firing in tonic firing mode (b) during wakefulness. The parameters' values are the same as on the Fig.5.11.

along the sleep-wake cycles. These neurons, according to experimental data, are not going into a silent state during sleep but generate regular burst discharges. During wakefulness they demonstrate high-frequency, single-spike activity.

The functionally relevant changes of synchronization activity at these transitions can best bee seen from the direct comparison of voltage traces of both neurons during sleep and wake states. Figure 5.12 demonstrates such comparison. These diagrams show that the bursts of the two neurons, and even the individual spikes within the bursts, always appear at almost identical times (a). Such coincidences do not exist in the tonic firing mode (b). In these simulations, the transitions of thalamic neurons from synchronized bursting activity to asynchronous single spike activity, can be recognized without the need for phase plots. These transitions are introduced by the input from the hypocretin neuron which goes from a silent to a firing state due to the homeostatic mechanisms with circadian drive. The principle alterations of firing patterns and synchronization states in hypothalamic as well as thalamic neurons perfectly fit to the experimental data.

Chapter 6

DISCUSSION

This study presents a mathematical model of sleep-wake cycles which is based on a novel concept of homeostatic regulation of sleep. In the focus of this concept are the state-dependent alterations of impulse generation and synaptic transmission of hypocretin/orexin neurons in the lateral hypothalamic area (LHA). The functionally relevant dynamics have been ascribed to spike-dependent attenuation of the synaptic efficacy of the neuropeptide hypocretin which is superimposed with a continuous recovery process.

These mechanisms have been illustrated with a minimal model, which considers neurons and synapses and allows to simulate the effects of such external disturbances as alarm clock, sleep deprivation or the interruption of wakefulness by a nap. In connection with a circadian input, the model can account for the typical alterations of the firing states of hypocretin neurons according to regular sleep-wake cycles. With synaptic projections from hypocretin to thalamic neurons, it also mimics the corresponding transitions of thalamic neurons from asynchronous to synchronized activity states.

In the following sections, the physiological background of this novel concept will be addressed, followed by a discussion of its mathematical realization with a simplified but physiologically justified mechanism-based approach. This approach will then be compared with existing concepts and models of sleep regulation. Finally, future model extensions and suggestions for experimental evaluation of the proposed concept will be described.

6.1 Concept

The concept of a homeostatic sleep-wake process has been developed in reference to experimental and clinical data which suggest that the neuropeptide hypocretin plays an essential role in sleep-wake regulation (Sutcliffe and de Lecea, 2002; Tsujino and Sakurai, 2009). From experimental studies, it is well established that hypocretin neurons are firing during wakefulness while they are almost silent during sleep (de Lecea et al., 1998; Sakurai et al., 1998; Lee et al., 2005; Mileykovskiy et al., 2005). Moreover, lack of hypocretin or its receptors leads to the sleep disorder narcolepsy (Lin et al., 1999; Chemeli et al., 1999; Thannickal et al., 2000; Gerashchenko et al., 2001), while its intravenous or nasal administration improves wakefulness and alertness of sleep deprived subjects (Deadwyler et al., 2007). These and other data (see Chapters 1 and 2) imply that hypocretin is crucial for wakefulness and that impaired hypocretin transmission induces sleepiness. The question was, whether and how such alterations of hypocretin effects could also contribute to the homeostatic control of regular sleep-wake cycles.

Important hints in this context came from neurophysiological experiments which had demonstrated that hypocretin neurons are reciprocally connected with other excitatory neurons, including local glutamate interneurons in the LHA (Li et al., 2002). It has been suggested that these positive feedback loops support firing activity of the hypocretin neurons. Accordingly, transitions from wakefulness to sleep, which are going along with transitions from firing to silence of hypocretin neurons, should be associated with an impairment of such reciprocal excitatory feedback loops. This can be achieved in different ways, e.g. by external influences like inhibitory inputs to the hypocretin neurons or their partners. However, such an assumption would simply shift the problem to other brain nuclei where no really promising candidates for a homeostatic process could be seen (see section 1.3).

Therefore, the focus of this study was laid on possible homeostatic mechanism which may occur inside the positive feedback loops of hypocretin neurons with others. In search for state-dependent alterations in synaptically connected neuronal circuits it is reasonable to evaluate the possible effects of synaptic plasticity. This physiologically well known process is often observed with regard to learning and memory but has only occasionally been discussed in context with homeostatic sleep functions (Tononi and Cirelli, 2006). Moreover, synaptic plasticity is mostly

attributed to the classical transmitters, such as glutamate, while here it is ascribed to the synaptic effects of a neuromodulator, the neuropeptide hypocretin.

The introduction of activity-dependent plasticity of hypocretin synaptic transmission as a mechanism for homeostatic regulation of sleep-wake cycles is a completely new approach. It is fully consistent with the well known hypocretin effects, especially with the fact that hypocretin is required to maintain wakefulness while its lack introduces sleepiness. Similar effects may occur in the course of regular sleep-wake cycles. In this case they are not attributed to a pathological disturbance of hypocretin transmission but are the result of a physiological process of synaptic plasticity.

Synaptic plasticity is a ubiquitous feature and has already been reported to occur during sleep deprivation in the form of synaptic potentiation at glutamatergic synapses on hypocretin neurons (Rao et al., 2007). From a superficial point of view these results may appear contradictory to the proposed concept of synaptic weakening during wakefulness. However, the synapses which have been examined by Rao et al. are the synapses ON the hypocretin neurons, while the synaptic plasticity in this model appears at the synapses OF the hypocretin neurons. Considering these differences, the results of Rao and colleagues perfectly fit to the here described concept. The strengthening of the glutamatergic synapses on the hypocretin neurons can help to keep them firing during sleep deprivation despite the homeostatic depression of the hypocretinergic synapses. In an extended model version, such mechanisms could replace the additional current input which has been used for the simulation of sleep deprivation in Figure 5.5.

Similar effects can be expected from synaptic potentiation in thalamocortical circuits during wakefulness which has been hypothesized by Tononi and Cirelli (2006). The strengthening of synaptic transmission among thalamic and cortical neurons could be a functionally important mechanism to keep them in an alert state over the day when the homeostatic drive from the hypocretin neurons is decreasing.

It can be expected that many more processes are involved in the regulation of sleep-wake cycles which, together, constitute a system of high flexibility. Even the lack of most relevant factors of sleep-wake regulation can, obviously, be compensated by other mechanisms. This has been shown for hypocretin as well as for adenosine, which may contribute to sleep-wake regulation as an additional, homeostatic sleep substance (Basheer et al., 2004; Landolt, 2008). Neither hypocretin

knockout mice nor others which are lacking the A1 adenosine receptors displayed significant disturbances of the homeostatic sleep markers (Mochizuki et al., 2004; Stenberg et al., 2003). Only the sleep and wake states of hypocretin knockout mice became unstable, which may be taken as an indication that the omission of hypocretin cannot fully be compensated.

6.2 Modeling approach

The concept of homeostatic hypocretin effects that are manifested in neuronal circuits with reciprocal excitation has been implemented in a mathematical model. This model also considers the modulation of homeostatic hypocretin effects by a circadian input and the possible impact of hypocretin neurons on thalamic synchronization.

The complete model consists of only four neurons (Figure 4.1). There are two hypothalamic neurons which are reciprocally coupled by excitatory chemical synapses. One of them represents the hypocretin neurons and the other one the local glutamate interneurons. Thalamic activity is represented by two gap junction coupled neurons, receiving synaptic input from the hypothalamic hypocretin neuron. All external influences are comprised in a compound current input to the hypocretin neuron. An additional noise current is only added to the thalamic neurons to allow de-synchronization out of a synchronized state.

Admittedly, these are significant simplifications, especially in considering synaptic interactions between single neurons where we physiologically have to expect neuronal networks of significant heterogeneity. However, in this modeling study, major emphasis is laid on the examination of the principle dynamics which can develop from neuronal interactions with synaptic plasticity. Therefore, the focus was on the synaptic mechanisms and accompanying alterations of spiking activity. In this situation the physiological mechanisms can be examined and illustrated on the level of individual neurons and synapses much better than with compound action potentials or mean firing rates. With the later some relevant information, e.g. the alterations of thalamic impulse pattern, would be smoothed out.

The individual model neurons and the synapses have been implemented with a mechanism-based, Hodgkin-Huxley type approach which means that relevant dynamics develop from activation and inactivation of voltage- and transmitter-

gated ion channels. Further simplifications have been implemented with regard to voltage-dependent ion channels in using an only minimal set of equations.

Both hypothalamic neurons were simulated with the simplest Hodgkin-Huxley type model neuron. The voltage-dependent currents include only sodium and potassium currents for spike generation. Nevertheless, these model neurons can be tuned from silent to firing states of different frequencies according to sleep and wake states. As a consequence of these simplifications, the model is extraordinary robust against external disturbances as long as it operates away from the bifurcation point where the transitions occur. The implementation of more complex neurons and/or the addition of noise can be used to modify the network's robustness and sensitivity but should not destroy the principle dynamics which have been elucidated in this study.

The thalamic neurons comprise two additional voltage-dependent currents which are activated at lower membrane potentials than the spike-generating currents, but have longer time constants of activation. These currents are for the generation of slow subthreshold membrane potential oscillations. The complete model neuron, i.e. the slow subthreshold currents together with the spike-generating currents, constitute a flexible single-neuron pattern generator which can be tuned to different activity states (for details see Braun et al., 2003a,b). These include generation of impulse groups (burst discharges) and pacemaker-like single-spike activity (tonic firing) which are the functionally relevant firing patterns of thalamic neurons with regard to sleep and wakefulness (McCormick and Feeser, 1990).

In this model the full variety of the voltage-dependent ionic currents in the hypothalamic and thalamic neurons is not considered. The details, anyhow, are still under debate and far from being fully discovered and understood. The focus of this study was on a better understanding of principle mechanisms, i.e. the transitions from silence to firing of hypocretin neurons and from unsynchronized tonic activity to synchronized burst discharges of thalamic neurons. Therefore, the detailed set of ionic currents was not required.

As an additional simplification the thalamic neurons are coupled only by gap junctions. This choice, however, is justified as it has experimentally been demonstrated that gap junction coupling plays a crucial role for the synchronization of thalamocortical networks (Landisman et al., 2002; Fuentealba et al., 2004).

While the gap junction currents are modeled in the general simple form, the transmitter-gated current have been implemented in more details, although,

again simplified. The primary goal was to obtain physiologically realistic postsynaptic currents and potentials due to activation of ionotropic glutamate and metabotropic hypocretin receptors. This have been achieved by a mechanisms-based approach which allows synaptic modulation with physiologically appropriate parameters adjustment.

Particular efforts have been invested to the simulation of the activity-dependent decline of hypocretin effects. The reason is, as discussed above, that there is not yet an experimental proof for the proposed effects while such effects, principally, can be induced in different ways, e.g. by a decrease of presynaptic transmitter availability or by the internalization of postsynaptic receptors. The algorithms of the actual model version can account for both mechanisms and allow to simulate many more effects of synaptic modulation, e.g. by receptor agonists and antagonists or re-uptake inhibitors. The development of these algorithms can attain particular importance when the effects of drug administration have to be modeled.

Compared to the specific properties of the synaptic algorithms it is almost neglectable that all synaptic equilibrium potentials, for simplicity, have been set to the Na-equilibrium potential. It would not be a problem to achieve the same results with other equilibrium potentials. Changing the equilibrium potential means, first of all, change of the "driving force". The synaptic current could be readjusted, for example, by the maximum conductances of the corresponding equations. The ionic mechanisms of synaptic hypocretin effects are, anyhow, not yet fully understood. It is still under debate whether hypocretin induces postsynaptic depolarization through the inhibition of potassium channels (Xia et al., 2005) or acts via the opening of sodium and/or calcium channels (Eriksson et al., 2001).

The circadian input is modeled in form of a gradually changing current which may correspond to a compound current input. Such a representation of a neuronal population, otherwise, has been avoided in favor of spiking neurons. In the actual model, where the focus is on the homeostatic mechanisms, this disagreement with the principle concept can be accepted, especially as the circadian process is only unidirectionally coupled. There are, so far, no reciprocal inputs to the circadian pacemaker to be analyzed on a mechanism-based level of ionic conductances. The circadian input significantly interferes with the homeostatic dynamics but this happens at the hypocretin neuron.

The implementation of a homeostatic process on the basis of physiological mechanism makes the major difference between this model and other neuronal models of sleep-wake transitions. Moreover, it is not the classical transmitter glutamate but a co-transmitter, the neuropeptide hypocretin, to which the key role has been assigned.

6.3 Comparison to other models of sleep regulation

The basic idea and modeling approach of this study is significantly different from the already existing concepts and models of sleep-wake regulation. Nevertheless, it is not contradictory to others but provides a mechanism-based explanation where the conceptional models, e.g. the two-process model of Borbély (1982) and the flip-flop concept of Saper et al. (2005), remain on the level of formal or phenomenological description. In comparison to the already existing neuronal models, the actual model can be considered as a supplementary approach, especially in focusing on the homeostatic process of sleep-wake regulation which, so far, has not been described on a mechanism-based level but mostly was implemented in a rather general form, if considered at all.

6.3.1 Two-process model

The basic concept of modeling the sleep-wake cycles on the basis of the homeostatic process which is driven by the circadian process is closely related to the two-process model (Borbély, 1982; Daan et al., 1984; Achermann, 2004). There are obvious similarities with regard to the circadian process which here is implemented in the same form as in the two-process model (see $I_{circadian}$). Also the homeostatic variable M exhibits a time course similar to that of the homeostatic process S (see section 1.2.1). It does not make a principle difference that M is decreasing during the wake state while S is increasing. An increasing sleep drive can also be considered as a decreasing wake drive.

The major difference is, that the previously formal description of the homeostatic process can now be related to physiological mechanisms. While the homeostatic sleep process S of the two-process model is implemented as an explicit

exponential function, the corresponding variable M of the here presented model develops from specific neuronal mechanisms, i.e. the activity-dependent changes of synaptic efficacy of the neuropeptide hypocretin.

The complex interrelations between the diverse variables of the neuronal model lead to additional, functionally significant differences. In the neuronal model decline and recovery of the homeostatic process are closely interrelated. Both depend on the actual level of synaptic efficacy M, which is determined by the firing rate of the hypocretin neuron. This is the reason why the circadian pacemaker in this model is not simply delivering the thresholds for the sleep-wake transitions as it does in the two-process model. It is continuously modulating the firing state of the hypocretin neuron and, thereby, modulates the effective synaptic strength at the glutamate and thalamic neurons. The firing rate of the glutamate neuron, in turn, influences firing of the hypocretin neuron and introduces further alterations of synaptic transmission. Likewise, any additional external input, e.g. from other sleep-wake related systems, will interfere with the complete system dynamics, irrespective of whether such connections are made by simple current injection, as for the circadian pacemaker, or via synapses.

6.3.2 The flip-flop model

The flip-flop model of Saper et al. (2005) considers reciprocal inhibition between sleep-promoting neurons in the ventrolateral preoptic area (VLPO) and wake-promoting monoaminergic neurons in the brain stem. The hypocretin neurons in the lateral hypothalamus are proposed to stabilize the states of sleep and wakefulness by having excitatory projections to the monoaminergic neurons and inhibitory from the VLPO (see Figs. 1.2 and 1.4). This concept essentially recapitulates experimental findings which demonstrated that the VLPO predominates during sleep, thereby, inhibiting the monoaminergic neurons, while the monoaminergic neurons are active during wakefulness and inhibiting the VLPO. Activity of monoaminergic neurons during wakefulness is strengthened by the excitatory input from the wake-active hypocretin neurons which is absent during sleep because the hypocretin neurons are blocked by the inhibitory input from the VLPO.

The flip-flop concept only describes the situation during the sleep and wake states, but does not explain the cause of the transitions. The neuronal model that is presented here provides an explanation on the basis of homeostatic hypocretin

effects which is in full agreement with the flip-flop concept.

During wakefulness, when the hypocretin neurons are firing, they are keeping the monoaminergic neurons in an active state which, in turn, suppress the VLPO neurons. When the synaptic efficacy of the hypocretin neurons is decreasing, also their excitatory synaptic input to the wake-promoting monoaminergic neurons will decrease. The firing rate of the monoaminergic neurons and, hence, their inhibitory input to the VLPO neurons will be reduced. The VLPO neurons, thereby, can increase their firing rate what increases the inhibitory input to the LHA. When the homeostatic circuit is already weakened the inhibitory input from the VLPO can facilitate the transition of hypocretin neurons to a silent state, i.e. the transition to sleep.

During the sleep state the hypocretin synaptic efficacy can recover. Once it is sufficiently recovered wakefulness can be re-introduced by the circadian or any other excitatory input which is strong enough to activate the homeostatic circuit. The hypocretin neurons will activate the monoaminergic neurons. These will suppress the activity of the VLPO neurons, thereby, removing the inhibitory input to the hypocretin neurons and further facilitating their firing, i.e. the transition to wakefulness. In this way, the phenomenological description of sleep-wake states by the flip-flop concept can be related to neuronal mechanisms which allow to account for the transitions between the different states.

6.3.3 Mechanism-based models

There is a diversity of modeling approaches which are based on similar ideas as the here described concept. The common approach is the understanding of some specific phenomena of sleep-wake regulation by the examination of systemic interactions between sleep-wake related functions, brain areas and/or transmitter systems (Chou, 2003; Tamakawa et al., 2007; Phillips and Robinson, 2007; Best et al., 2007; Diniz Behn et al., 2007; Diniz Behn et al., 2008).

Compared to the actual study, some of these models are more detailed, others consider more sleep-wake functions or brain areas and many of them can successfully simulate a higher diversity of sleep-wake phenomena, including the ultradian structure of sleep. However, the homeostatic process in these models is typically realized as an explicit state-dependent function mostly assuming, similar to the original two-process model, the accumulation of a sleep substance. With this

respect, the actual study can provide a perfect extension also for the more generalized neuronal models. The more interesting aspect, however, concerns the question how the different modeling approaches at the micro- and macroscopic scale can be interrelated.

The existing models of thalamic activity (Bazhenov et al., 2002; Hill and Tononi, 2005), are usually very detailed and include thousands of neurons and synapses. The neurons are modeled by a conductance-based approach, i.e. with voltage- and transmitter-gated ion channels, similar to that used in the present study. However, physiologically relevant changes in these thalamocortical models are induced from outside, i.e. by formal external functions that are used to simulate the input from the neuromodulatory regions. The focus of these models is laid on the thalamocortical interactions. Therefore, subthalamic brain areas, like the hypothalamus, are not considered, although their impact on sleep-wake regulation is well demonstrated.

With this respect, the here described concept of hypothalamic sleep-wake regulation, although modeled with individual neurons instead of large neuronal networks, could provide a valuable extension of the thalamocortical models. Furthermore, the extension of the hypothalamic model by excitatory projections from the hypocretin neuron to the previously developed models of thalamic neurons demonstrates that the sleep-wake related changes of thalamic activity can simply be induced by an external current. None of the otherwise implemented changes of ionic conductances or synaptic connectivity are required.

Accordingly, the reasons for this particular behavior have to be searched in the specific properties of the neuronal model which here has been implemented for the simulation of thalamic activity. This model, originally developed for the simulation of cold receptor discharges (Braun et al., 2003a), constitutes a paradigmatic, most flexible single-neuron pattern generator (Braun et al., 2003b) with particular synchronization properties (Postnova et al., 2007a,b). With current injection, as with many other parameter changes, these model neurons can be tuned through a diversity of impulse patterns. These include burst discharges and pacemaker-like tonic firing corresponding to the dominant patterns in thalamocortical circuits during sleep and wakefulness, respectively. The neurons tuned to different activity patterns exhibit significantly different response properties which actually are under further examination (Finke et al., 2008, Postnova et al., in print a,c). Most notably, for some not yet fully understood reasons, these model neurons

hardly synchronize in the tonic firing mode while they easily synchronize in the bursting. This perfectly reflects the experimentally recorded changes of activity in thalamocortical circuits along the sleep-wake transitions (McCormick and Feeser, 1990)

6.4 Outlook

This model has been developed with major emphasis on the simulation of homeostatic effects which have been related to state-dependent alterations of the synaptic efficacy of the neuropeptide hypocretin. Besides of a circadian input and projections to thalamic neurons, it does not yet consider the interactions with other sleep-wake related functions. With this respect, this novel concept and its mathematical implementation is only a first but, nevertheless, promising step towards a more comprehensive concept of sleep-wake regulation.

Indeed, it was a major motivation for the development of a sleep model on the basis of neurons and synapses to allow further model extensions on the level of physiological interactions. The model is built in such way that the functionally relevant changes of the model variables, e.g. voltages or currents, have their physiological correlates and are experimentally accessible. At the same time, the simulations outcomes should provide the relevant changes according to behavioral states (alertness, sleep intensity) which are accessible by conventional clinical measures, e.g. by the amplitude and frequency of EEG waves. The following sections will describe ideas of future model extensions and suggestions for further experimental evaluation of the proposed concept.

6.4.1 Model extensions

Model extensions can already be made on the basis of the actual circuits, for example, by the introduction of stochastic components in the hypothalamic circuit. Implementation of appropriate noise terms can account for endogenous randomness of the biological systems, e.g. ion channels opening/closing and synaptic transmitter release. On the other hand, noise can account for random changes of environmental input, which can increase arousal or induce sleepiness.

Neuronal and synaptic noise will lead to more smooth transitions of the individual neurons' responses on external stimuli (direct or synaptic currents), thereby,

broadening and flattening their sensitivity range (Huber and Braun, 2006; Finke et al., 2008). Similar effects can be expected when a heterogeneous network of multiple of neurons instead of single neurons is implemented (Tessone et al., 2006). In any case, the flip-flop like sleep-wake transitions, i.e. switching between silence and firing of the hypothalamic circuits, will remain and, accordingly, also the abrupt transitions of thalamic activity pattern and synchronization states. Such switching is a property of the reciprocal excitatory feedback loop.

The impact of physical and social stimuli, which often are the determinant factors for sleep-wake behavior, can be comprised in such noise terms. It can also be examined more specifically with additional current inputs at specific phases of the day or during the night, similar to the examples with napping, sleep deprivation and the effects of alarm clock (Figs. 5.5 and 5.6).

Regular changes of the environment, like the day-night cycles, can be considered by additional current terms of corresponding time courses. Light-dark effects can be interlinked with the inhibition and stimulation of melatonin release from the pineal gland which can then be connected to the circadian pacemaker. Such extensions are specifically required for the examination of (1) the adjustment of the internal circadian clock to the external day-night cycles and (2) de-synchronization of the sleep-wake cycles, e.g. in shift workers or after jet lag. For the evaluation of the physiologically relevant circadian processes it is essential to replace the circadian current input of the actual model by a mechanism-based simulation of the SCN neurons with appropriate alterations of the firing rate. Further it can be connected to a mechanism-based model of melatonin release.

The circadian clock, besides of its impact on sleep-wake cycles, is also modulating a diversity of autonomic functions such as energy control, temperature regulation and hormone secretion (for review see Dijk and Edgar, 1999; Kalsbeek et al., 2006). The circadian alterations of the these functions, in turn, are intimately related to the sleep-wake cycles as it is indicated, for example, by a strong increase of growth hormone release in the first part of the night and pronounced cortisol peaks before awakening (Czeisler and Edgar, 1999).

Moreover, there are close interlinks between the autonomic functions, including sleep-wake control, and the limbic system (Hockman and Thomas, 1972). These interdependencies can particularly well be seen in pathological states, most clearly in mood disorders which are often associated with significant disturbances of autonomic functions (for review see Tsuno et al., 2005). Especially major depressive

disorder is frequently accompanied by sleep disturbances as well as by increased cortisol levels (Brown et al., 2004). The implementation of such a manifold of interactions between sleep-wake control, autonomic and emotional functions would require enormous efforts, especially as the cellular mechanisms of many of the above mentioned functions and their disturbances are far from being fully understood.

Therefore, priority shall be given to the examination of the functional connections with those brain nuclei which change their activity in direct relation to the sleep and wake states but are not yet considered in the actual model. These are, as indicated in Figure 1.2, the ventrolateral preoptic nuclei of the hypothalamus (VLPO) and the monoaminergic and cholinergic nuclei of the arousal system.

The contribution of the VLPO to sleep-wake regulation in supplement to homeostatic hypocretin effects have already been discussed in the previous section in context with the flip-flop concept (Saper et al., 2005). Both, the VLPO GABAergic and the LHA hypocretinergic neurons can additionally be examined as possible targets of the so-called "sleep substance" adenosine (see section 1.3.1). The concentration of adenosine, as a byproduct of energy metabolism, was proposed to increase during the active wake state and decrease during sleep. Indeed, accumulation and decline of adenosine have been recorded in some brain areas where it exerts inhibitory effects. Accordingly, the accumulation of adenosine during the day can directly inhibit hypocretin neurons, thereby, reducing their firing rates (Liu and Gao, 2007). Simultaneously, as proposed by Morairty et al. (2004), it can indirectly activate the VLPO which would lead to an additional inhibition of hypocretin neurons through the inhibitory projections from the VLPO. In this way, the implementation of adenosine should additionally strengthen the above described effects of the interaction between the VLPO and the LHA, including the monoaminergic nuclei, i.e stabilizing the actual sleep or wake state but facilitating state-transitions once they have started.

A complete model of sleep-wake regulation also needs to consider the diurnal structure of sleep, i.e. the ultradian transitions between REM and NREM sleep. These transitions appear due to the reciprocal connections between monoaminergic REM-off and cholinergic REM-on nuclei which are receiving additional inputs from the LHA and the VLPO (McCarley and Hobson, 1975; Pace-Schott and Hobson, 2002; Lu et al., 2006b). To simulate REM-NREM transitions the presented model has to be extended to include these interactions, and, eventually, to

consider the mechanisms of the homeostatic regulation of REM sleep (Brunner et al., 1990).

6.4.2 Suggestions for experiments

This modeling study is based on a novel concept of homeostatic sleep-wake regulation which is attributed to synaptic plasticity of hypocretin neurons. Synaptic plasticity is a well known phenomenon which was observed during sleep deprivation (Rao et al., 2007) and has been hypothesized to appear in thalamocortical circuits (Tononi and Cirelli, 2006). In both cases synaptic plasticity appeared in form of synaptic potentiation. Activity-dependent attenuation of hypocretin transmission is proposed here for the first time and, accordingly, has not yet been examined experimentally.

Conclusive experiments may be performed similar to those of Rao et al. (2007), however, with a major difference, namely that the recordings should not be made at hypocretin neurons but at neurons which are receiving hypocretin input. As such kind of experiments shall best be done in brain slices, it is reasonable to make the recordings from local interneurons in the LHA where also the hypocretin neurons are located. Otherwise, hypocretin-induced synaptic potentials would be missing.

These studies must not necessarily be done with exactly the same experimental design as used by Rao et al. for the examination of synaptic plasticity after sleep deprivation. Such experiments require enormous efforts for statistical analysis which means sacrificing a high number of rats, preceded by drug treatment or appropriate handling. In search for synaptic depression of hypocretin effects there can be even more difficulties for several reasons. Firstly, it can be expected that the identification of hypocretin-innervated neurons will be more difficult than that of hypocretin neurons for which specific markers exist. Secondly, the alterations of synaptic efficacy of a neuropeptide like hypocretin will not so easily be recognizable in individual postsynaptic potentials as those of glutamate where even the effects of AMPA and NMDA receptors can be distinguished.

As an alternative, synaptic changes can, eventually, be detected in acute brain slice recordings during spontaneous firing of the hypocretin neurons. However, as the synaptic depression is proposed to develop very slowly under natural conditions, i.e. over wake periods of several hours, it would be of advantage if a presy-

naptic hypocretin neuron could be identified together with the interneuron from which the recordings are made. When the presynaptic hypocretin neuron could be subjected to high-frequency stimulation it might become easier to see the proposed alterations of postsynaptic hypocretin effects within reasonable recording time, i.e. within the life time of brain slices. To be sure that hypocretin effects are recorded, control experiments with hypocretin application or, even better, with application of a hypocretin antagonist, would be helpful.

In case that the suggested decrease of the synaptic hypocretin transmission can experimentally be confirmed, further experiments can be designed to distinguish between the proposed alternatives of presynaptic transmitter depletion and postsynaptic receptor internalization. In any way, electrophysiological experiments that can provide conclusive information about synaptic plasticity in relation to a neuropeptide, like hypocretin, will not be an easy task to do.

Electrophysiological experiments with regard to the proposed synaptic plasticity of hypocretin transmission can be complemented by clinical studies in order to evaluate the impact of hypocretin in normal sleep homeostasis, which can be measured by amplitude of slow waves in sleep EEG. Similar to examinations of adenosine effects on sleep homeostasis by application of the adenosine antagonist - caffeine (Landolt et al., 2004; Drapeau et al., 2006; James and Keane, 2007), such studies could best be done with application of a hypocretin antagonist. However, while the adenosine antagonist caffeine is a common drug and can easily be applied, antagonists for the only recently discovered neurotransmitter hypocretin are not yet easily available.

The promising substance is already in development by pharmacological companies who search for medical treatment of insomnia. However, this substance is still in a clinical trial stage and is not yet generally provided for basic research. Nevertheless, there is reliable information that hypocretin antagonists can induce somnolence and increase the amount of REM and NREM sleep (Brisbare-Roch et al., 2007). Such data additionally support the concept of hypocretin as major substance in sleep-wake control.

6.5 Conclusion

With this study a novel concept of homeostatic sleep regulation has been proposed and transformed in a mathematical model of sleep-wake regulation. The basic assumptions have been described and discussed together with the advantages and simplifications of the mechanism-based approach.

The simulation results strongly support the principle idea of activity-dependent alterations of synaptic hypocretin effects as the source of homeostatic sleep mechanisms. It has been demonstrated that a simple extension of the homeostatic model by a circadian current input leads to regular alterations between silence and firing of hypocretin neurons on a real time scale with physiologically appropriate time relations between sleep and wakefulness. Moreover, connecting this hypothalamic model of sleep-wake regulation with a previously developed model of thalamic neurons also provides an explanation for the associated changes of impulse pattern and synchronization states in thalamic circuits.

These simulations, apart from the actual results, demonstrate a major advantage of such a physiologically motivated modeling approach, namely that it easily can be combined with other mechanism-based models and can also be related to more formal concepts of sleep-wake regulation. Accordingly, major parts of the discussion have been devoted to illustration of the relations between the here described mechanisms and, for example, the formal two-process model or the flip-flop concept. Especially, a diversity of future model extensions have been addressed for the involvement of other relevant mechanisms of sleep-wake regulation, e.g. the effects of adenosine or the impact of external stressors. Finally, some ideas for electrophysiological and clinical studies of the proposed concept have been described.

Altogether, this modeling approach has important advantages: 1) it can be extended to examine the effects of additional internal and external stimuli or physiological parameters, 2) it can be connected to other brain areas which exhibit sleep-wake related activity changes, and 3) it can easily be adapted to new experimental discoveries. In conclusion, it should allow to bring together a manifold of experimental and clinical data from different functional levels into a comprehensive, neuron-based model towards a better understanding of sleep-wake regulation.

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Verzeichnis der akademischen Lehrer

Meine akademischen Lehrer waren Damen/Herren

... in Marburg

Besedovsky, Braun, Hemmeter, Huber, Mandrek, McGregor, Penzel, del Rey, Voigt, Walter.

... in Lyngby

Mosekilde, Sosnovtseva.

... in Saratov

Akchurin, Alekseenko, Anischenko, Astahov, Berezina, Bezruchko, Chernova, Chetverikov, Debrov, Fedorova, Fillinova, Galanzha, Hlebcov, Hohlov, Ignatiev, Karasev, Karcev, Khovanov, Klimshin, Krasilnikov, Kochubej, Kossovich, Kulikov, Kvashnin, Listov, Maksimova, Martynovich, Melnikov, Ovchinnikov, Pavlov, Pravdin, Pravdina, Poplavskij, Postnov, Pozdneva, Razumovskaya, Ryabuho, Salij, Sevostiyanov, Shabunin, Shevcov, Simonenko, Sinichkin, Tuchin, Tuchin, Vadivasova, Vdovina, Venig, Voronina, Uliyanov, Zimnyakov.

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