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Neurobiology of REM and NREM sleep ☆

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Abstract

This paper presents an overview of the current knowledge of the neurophysiology and cellular pharmacology of sleep mechanisms. It is written from the perspective that recent years have seen a remarkable development of knowledge about sleep mechanisms, due to the capability of current cellular neurophysiological, pharmacological and molecular techniques to provide focused, detailed, and replicable studies that have enriched and informed the knowledge of sleep phenomenology and pathology derived from electroencephalographic (EEG) analysis. This chapter has a cellular and neurophysiological/neuropharmacological focus, with an emphasis on rapid eye movement (REM) sleep mechanisms and non-REM (NREM) sleep phenomena attributable to adenosine. The survey of neuronal and neurotransmitter-related brainstem mechanisms of REM includes monoamines, acetylcholine, the reticular formation, a new emphasis on GABAergic mechanisms and a discussion of the role of orexin/hypcretin in diurnal consolidation of REM sleep. The focus of the NREM sleep discussion is on the basal forebrain and adenosine as a mediator of homeostatic control. Control is through basal forebrain extracellular adenosine accumulation during wakefulness and inhibition of wakefulness-active neurons. Over longer periods of sleep loss, there is a second mechanism of homeostatic control through transcriptional modification. Adenosine acting at the A1 receptor produces an up-regulation of A1 receptors, which increases inhibition for a given level of adenosine, effectively increasing the gain of the sleep homeostat. This second mechanism likely occurs in widespread cortical areas as well as in the basal forebrain. Finally, the results of a new series of experimental paradigms in rodents to measure the neurocognitive effects of sleep loss and sleep interruption (modeling sleep apnea) provide animal model data congruent with those in humans.
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Keywords: REM; NREM

1. Introduction

This paper presents an overview of the current knowledge of the neurophysiology and cellular pharmacology of sleep mechanisms. It is written from the perspective that recent years have seen a remarkable development of knowledge about sleep mechanisms, due to the capability of current cellular neurophysiological, pharmacological and molecular techniques to provide focused, detailed, and replicable studies that have enriched and informed the knowledge of sleep phenomenology and pathology derived from electroencephalographic (EEG) analysis. This chapter has a cellular and neurophysiological/ neuropharmacological focus, with an emphasis on rapid eye movement (REM) sleep mechanisms and non-REM (NREM) sleep phenomena attributable to adenosine. A detailed historical introduction to the topics of this chapter is available in the Steriade and McCarley book [1]. For the reader interested in an update on the terminology and techniques of cellular physiology, one of the standard neurobiology texts can be consulted (e.g., [2]). Overviews of REM sleep physiology are also available [1,3], as well as an overview of adenosine and NREM sleep [4]. The present paper

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draws on these accounts for the text, and we begin with brief and elementary overviews of sleep architecture and phylogeny/ontogeny to provide a basis for the later mechanistic discussions. Part I treats REM sleep and the relevant anatomy and physiology, and then comments very briefly on the role of hypocretin/orexin in REM sleep control. Part II discusses NREM sleep in the context of adenosinergic mechanisms.

Sleep may be divided into two phases. REM sleep is most often associated with vivid dreaming and a high level of brain activity. The other phase of sleep, NREM sleep or slow wave sleep (SWS), is usually associated with reduced neuronal activity; thought content during this state in humans is, unlike dreams, usually nonvisual and consists of ruminative thoughts. As one goes to sleep, the low voltage fast EEG of waking gradually gives way to a slowing of frequency and, as sleep moves toward the deepest stages, there is an abundance of delta waves, EEG waves with a frequency of 0.5 to <4 Hz and of high amplitude. The first REM period usually occurs about 70 min after the onset of sleep. REM sleep in humans is defined by the presence of low voltage fast EEG activity, suppression of muscle tone (usually measured in the chin muscles) and the presence, of course, of rapid eye movements. The first REM sleep episode in humans is short. After the first REM sleep episode, the sleep cycle repeats itself with the appearance of NREM sleep, and then about 90 min after the start of the first REM period, another REM sleep episode occurs. This rhythmic cycling persists throughout the night. The REM sleep cycle length is 90 min in humans and the duration of each REM sleep episode after the first is approximately 30 min. While EEG staging of REM sleep in humans usually shows a fairly abrupt transition from NREM to REM sleep, recording of neuronal activity in animals presents quite a different picture. Neuronal activity begins to change long before the EEG signs of REM sleep are present. To introduce this concept, Fig. 1 shows a schematic of the time course of neuronal activity relative to EEG definitions of REM sleep. Later portions of this chapter will elaborate on the activity depicted in this figure. Over the course of the night, delta wave activity tends to diminish and NREM sleep has waves of higher frequencies and lower amplitude.

2. REM sleep

REM sleep is present in all mammals, and recent data suggest that this includes the egg-laying mammals (monotremes), such as the echidna (spiny anteater) and the duckbill platypus. Birds have very brief bouts of REM sleep. REM sleep cycles vary in duration according to the size of the animal, with elephants having the longest cycle and smaller animals having shorter cycles. For example, the cat has a sleep cycle of approximately 22 min, while the rat cycle is about 12 min. In utero, mammals spend a large percentage of time in REM sleep, ranging from 50% to 80% of a 24-h day. At birth, animals born with immature nervous systems have a much higher percentage of REM sleep than do the adults of the same species. For example, sleep in the human newborn occupies two-thirds of time, with REM sleep occupying half of the total sleep time, or about one-third of the entire 24-h period. The percentage of REM sleep declines rapidly in early childhood

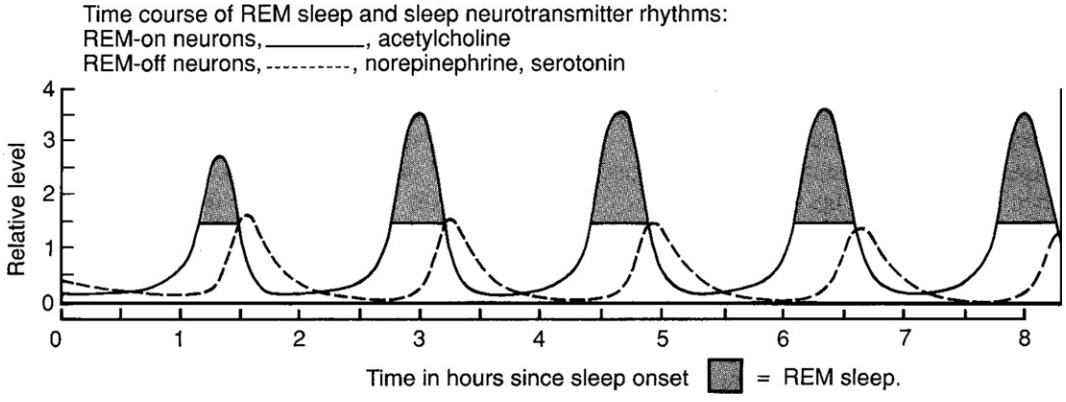

Fig. 1. Schematic of a night's course of REM sleep in humans showing the occurrence and intensity of REM sleep as dependent upon the activity of populations of "REM-on" (=REM promoting neurons), indicated by the solid line. As the REM-promoting neuronal activity reaches a certain threshold, the full set of REM signs occurs (black areas under curve indicate REM sleep). Note, however that, unlike the step-like EEG diagnosis of stage, the underlying neuronal activity is a continuous function. The neurotransmitter acetylcholine is thought to be important in REM sleep production, acting to excite populations of brainstem reticular formation neurons to produce the set of REM signs. Other neuronal populations utilizing the monoamine neurotransmitters serotonin and norepinephrine are likely REM-suppressive; the time course of their activity is sketched by the dotted line. The terms REM-on and REM-off quite generally apply to other neuronal populations important in REM sleep, including those utilizing the neurotransmitter GABA. These curves mimic actual time courses of neuronal activity, as recorded in animals, and were generated by a mathematical model of REM sleep in humans, the limit cycle reciprocal interaction model of McCarley and Massaquoi [96].

so that by approximately age 10 the adult percentage of REM sleep is reached, which is 20% of total sleep time. The predominance of REM sleep in the young suggests an important function in promoting nervous system growth and development.

Sleep with delta waves is minimally present in the newborn but increases over the first years of life, reaching a maximum at about age 10 and declining thereafter. Feinberg and co-workers [5] have noted that the first three decades of this delta activity time course can be fit by a gamma probability distribution and that a gamma distribution also fits the time course of synaptic density and frontal cortex metabolic activity, as measured by positron emission tomography (PET). They speculate that the reduction in these three variables may reflect a pruning of redundant cortical synapses that is a key factor in cognitive maturation, allowing greater specialization and sustained problem solving.

2.1. REM sleep physiology and relevant brain anatomy

2.1.1. REM-promoting systems

2.1.1.1. Transection studies. Lesion studies performed by Jouvet and co-workers in France demonstrated that the brainstem contains the neural machinery of the REM sleep rhythm (reviewed in [1]). As illustrated in Fig. 2, a transaction made just above the junction of the pons and midbrain produced a state in which periodic occurrence of REM sleep was found in recordings made in the isolated brainstem while, in contrast, recordings in the isolated forebrain showed no signs of REM sleep. Thus, while forebrain mechanisms

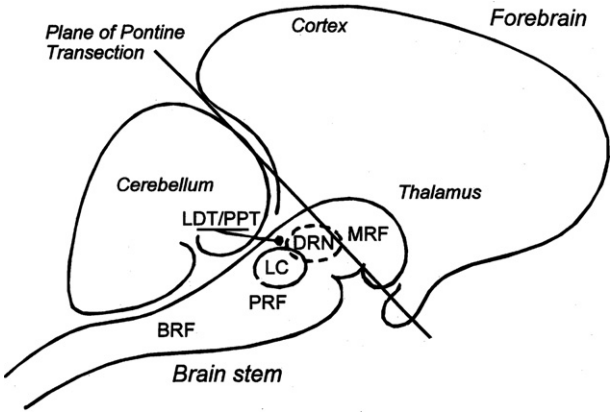

Fig. 2. Schematic of a sagittal section of a mammalian brain (cat) showing the location of nuclei especially important for REM sleep. *Abbreviations.* BRF, PRF, and MRF = bulbar, pontine, and mesencephalic reticular formation; LDT/PPT, laterodorsal and pedunculopontine tegmental nuclei, the principal site of cholinergic (acetylcholine-containing) neurons important for REM sleep and EEG desynchronization. LC, locus coeruleus, where most norepinephrine-containing neurons are located; RN, dorsal raphe nucleus, the site of many serotonin-containing neurons. The oblique line is the plane of transection that Jouvet [201] found preserves REM sleep signs caudal to the transection but abolishes them rostral to the transection.

(including those related to circadian rhythms) modulate REM sleep, the fundamental rhythmic generating machinery is in the brainstem, and it is here that anatomical and physiological studies have focused. The anatomical sketch provided by Fig. 2 also shows many of the cell groups important in REM sleep; the attention of the reader is called to the cholinergic neurons, which act as promoters of REM phenomena, and to the monoaminergic neurons, which act to suppress most components of REM sleep. Later sections will comment on GABAergic neurons, which are more widely dispersed rather than being located in specific nuclei. Note that Fig. 2 shows that the Jouvet transection spared these essential brainstem zones.

2.1.1.2. Effector neurons for different components of REM sleep: the brainstem reticular formation is principal location. By effector neurons we mean those neurons directly in the neural pathways leading to the production of different REM components, such as the rapid eye movements. A series of physiological investigations over the past four decades have shown that the "behavioral state" of REM sleep in nonhuman mammals is dissociable into different components under the control of different mechanisms and different anatomical loci. The reader familiar with pathology associated with human REM sleep will find this concept easy to understand, since much pathology consists of inappropriate expression or suppression of individual components of REM sleep. As in humans, the cardinal signs of REM sleep in nonhuman mammals animals are muscle atonia (especially in antigravity muscles), EEG activation (low voltage fast pattern, sometimes termed an activated or desynchronized pattern), and rapid eye movements.

PGO waves are another important component of REM sleep found in recordings from deep brain structures in many animals. PGO waves are spiky EEG waves that arise in the Pons and are transmitted to the thalamic lateral Geniculate nucleus (a visual system nucleus) and to the visual Occipital cortex, hence the name PGO waves. There is suggestive evidence that PGO waves are present in humans, but the depth recordings necessary to establish their existence have not been done. PGO waves are EEG signs of neural activation; they index an important mode of brainstem activation of the forebrain during REM sleep. It is worth noting that they are also present in nonvisual thalamic nuclei, although their timing is linked to eye movements, with the first wave of the usual burst of 3–5 waves occurring just before an eye movement.

Most of the physiological events of REM sleep have effector neurons located in the brainstem reticular formation, with important neurons especially concentrated in the PRF. Thus, PRF neuronal recordings are of special interest for information on mechanisms of production of these events. Intracellular recordings by Ito

et al. [6] of PRF neurons show that these effector neurons have relatively hyperpolarized membrane potentials and generate almost no action potentials during NREM sleep. PRF neurons begin to depolarize even before the occurrence of the first EEG sign of the approach of REM sleep, the PGO waves that occur 30–60 s before the onset of the rest of the EEG signs of REM sleep. As PRF neuronal depolarization proceeds and the threshold for action potential production is reached, these neurons begin to discharge (generate action potentials). Their discharge rate increases as REM sleep is approached and the high level of discharge is maintained throughout REM sleep, due to the maintenance of this membrane depolarization.

Throughout the entire REM sleep episode, almost the entire population of PRF neurons remains depolarized. The resultant increased action potential activity leads to the production of those REM sleep components which have their physiological bases in the activity of PRF neurons. PRF neurons are important for the following REM sleep phenomena: rapid eye movements, the generator for lateral saccades in the PRF; PGO waves controlled by a different group of neurons than rapid eye movements; and muscle atonia, controlled by a group of dorsolateral PRF neurons just ventral to the locus coeruleus. The muscle atonia neurons become active just before the onset of muscle atonia (see Section "PRF ventral to the locus coeruleus" for detailed discussion). Neurons in the midbrain reticular formation (MRF, see location in Fig. 2) are especially important for EEG activation, for the low voltage fast EEG pattern. These neurons were originally described as making up the ascending reticular activating system (ARAS), the set of neurons responsible for EEG activation. Subsequent work has enlarged this original ARAS concept to include cholinergic neurons, with contributions in waking to EEG activation also coming from monoaminergic systems, neurons utilizing serotonin and norepinephrine as neurotransmitters.

2.1.1.3. REM-ON neurons and REM promotion. Cholinergic mechanisms are important for the initiation and coordination of REM sleep. Current data suggest cholinergic influences act by increasing the excitability of brainstem reticular neurons important as effectors in REM sleep either directly or indirectly by disinhibition, by inhibiting GABAergic neurons which are inhibitory to reticular formation neurons. The essential data supporting cholinergic mechanisms are summarized below.

(1) Production of a REM-like state by direct injection of acetylcholine agonists into the PRF. It has been known since the mid-1960s that cholinergic agonist injection into the PRF produces a state that very closely mimics natural REM sleep (for review and detailed literature citations for this section, see [1]). The latency to onset and duration are dose-dependent; within the PRF, most workers have found the shortest latencies to come from injections in dorso-rostral pontine reticular sites. Muscarinic cholinergic receptors appear to be of major importance, with nicotinic receptors playing a lesser role. Of note, most of the *in vivo* cholinergic data has come from felines. In rats and mice, a similar REM induction effect can be induced, although it often is less robust in these species, perhaps as a result of difficulty in localization of applications in the smaller brains and interaction with circadian control (reviewed in [1]), as well as, perhaps, a different localization of GABAergic neurons inhibited by carbachol (see below). However, as described in the third point below, the *in vitro* evidence for carbachol excitatory effects on reticular formation neurons in the rat is undisputed. The precise site where *in vivo* carbachol is most effective in inducing REM or muscle atonia in the rat is disputed but appears to be within the PRF nucleus, pontis oralis (PnO) slightly rostral to the region just ventral to the locus coeruleus (subCoeruleus (SubC)) or in an area neighboring the superior cerabellar peduncle (ventral tegmental nucleus of Gudden (VTg)) [7–11]. Recent experiments using the acetylcholinesterase inhibitor neostigmine in the mouse suggest that the PnO is also an effective REM-inducing site in the mouse [12,13], although these findings have been disputed [14]. Of note also are the REM-reducing effects of muscarinic knockouts [15].

(2) LDT/PPT cholinergic projections to reticular formation neurons. Cholinergic projections in the brainstem and to brainstem sites arise from two nuclei at the pons-midbrain junction that contain cholinergic neurons: the laterodorsal tegmental nucleus (LDT) and the pedunculopontine tegmental nucleus (PPT). A sagittal schematic of their location is in Fig. 2. They project to critical PRF zones, as first shown by Mitani et al. [16] and repeatedly confirmed. A similar series of studies has documented the extensive rostral projections of cholinergic neurons to the thalamus and basal forebrain, where their actions are important for EEG activation, a topic to be discussed below.

(3) Direct excitation of PRF neurons by cholinergic agonists. In vitro pontine brainstem slice preparations offers the ability to apply agonists/antagonists in physiological concentrations, which are usually in the low micromolar range, whereas effective *in vivo* injections use concentrations that are a thousandfold greater, in the millimolar range, and, thus, raise the possibility of mediation of effects by nonphysiological mechanisms. Applications of micromolar amounts of cholinergic agonists *in vitro* in the rat produce an excitation of a majority (about two-thirds) of medial PRF reticular formation neurons. Another advantage of the *in vitro* preparation is the ability to use a sodium-dependent action potential blocker, tetrodotoxin; these experiments show that the excitatory effects of cholinergic agonists on PRF neurons in the rat *in vitro* are direct [17].

Furthermore, the depolarizing, excitatory effects of a cholinergic agonist mimic the changes seen in PRF neurons during natural REM sleep [6].

(4) LDT/PPT lesion and stimulation effects. Extensive destruction of the cell bodies of LDT/PPT neurons by local injections of excitatory amino acids leads to a marked reduction of REM sleep [18]. Low level (10 μA) electrical stimulation of LDT increases REM sleep [19]. On the other hand, Lu et al. [20] reported that ibotenic acid lesions of the LDT did not alter REM sleep while separate ibotenic acid lesions of the cholinergic PPT produced an increase in REM sleep, an effect they attributed to including part of the medial parabrachial nucleus.

(5) Discharge activity of LDT/PPT neurons across the REM cycle. A subset of these neurons has been shown to discharge selectively during REM sleep, and with the onset of increased discharges occurring before the onset of REM sleep [21–23] as schematized in Fig. 1. This LDT/PPT discharge pattern and the presence of excitatory projections to the PRF suggest that LDT/PPT cholinergic neurons may be important in producing the depolarization of reticular effector neurons, leading to production of the events characterizing REM sleep. The group of LDT/PPT and reticular formation neurons that become active in REM sleep are often referred to as REM-on neurons. Subgroups of PRF neurons may show discharges during waking motoric activity, either somatic or oculomotor, but a sustained depolarization throughout almost all of the population occurs only during REM sleep. Studies of the immediate early gene cFos expression have shown activation of ChAT-positive neurons in REM rebound in the rat following deprivation [24,25], although Verret et al. [26] did not. It must be emphasized that cFos expression, while useful, does not offer a 1:1 isomorphism with action potential occurrence (see [27]) Of particular note, single unit *in vivo* studies in the rat strongly support cholinergic activation during REM sleep (reviewed in [1]).

(6) Cholinergic neurons are important in the production of the low voltage fast (LVF) or "desynchronized" EEG pattern of both REM sleep and waking. Rostral projections of a subgroup of LDT/PPT neurons, those with discharges during both wakefulness and REM sleep, are important for the EEG activation of both REM sleep and waking (see extensive discussion in [1]).

2.1.1.4. Other neurotransmitters and PRF neurons. Peptides co-localized with acetylcholine. There are many peptides that are co-localized with the neurotransmitter acetylcholine in LDT/PPT neurons; this co-localization likely also means they have synaptic co-release with acetylcholine. The peptide substance P is found in about 40% of LDT/PPT neurons and, overall, more than 15 different co-localized peptides have been described. The role of these peptides in modulating acetylcholine activity relevant to wakefulness and sleep remains to be elucidated, but it should be emphasized that the co-localized vasoactive intestinal peptide (VIP) has been reported by a several different investigators to enhance REM sleep when it is injected intraventricularly. A later section of this chapter will discuss GABAergic influences, as well as the role of GABAergic reticular formation neurons.

2.1.1.5. REM muscle atonia. This is an important REM feature from a clinical point of view because disorders of this system are present in many patients with sleep disorders. Work by Chase and collaborators and by Segal and collaborators (reviewed in [1]) suggests three important zones for atonia, which we list according to their projections: pontine reticular formation –> bulbar reticular formation —> motoneurons. We here discuss only the PRF portion of the atonia circuitry.

PRF ventral to the locus coeruleus. Jouvet and colleagues in Lyon, France reported that bilateral lesions of the pontine reticular region just ventral to the locus coeruleus, termed by this group the perilocus coeruleus alpha, and its descending pathway to the bulbar reticular formation abolished the nuchal muscle atonia of REM sleep [28,29]. It is to be emphasized that this zone is a reticular zone, not one containing noradrenergic neurons like the locus coeruleus proper, and that the name refers only to proximity to the locus coeruleus. The Lyon group also reported that not only was the nuchal muscle atonia of REM suppressed, but that cats so lesioned exhibited "oneiric behavior," including locomotion, attack behavior, and behavior with the head raised and with horizontal and vertical movements "as if watching something". Morrison and collaborators [30] confirmed the basic finding of REM without atonia with bilateral pontine tegmental lesions but report that lesions extending beyond the locus coeruleus alpha region and its efferent pathway to bulb were necessary for more than a minimal release of muscle tone and for producing the elaborate "oneiric behaviors." The exact location and numbers of inhibitory pathways is still a matter of some controversy, with all investigators agreeing on the important, if not exclusive, role of the perilocus coeruleus alpha, or, as it is often termed, the subCoeruleus, SubC.

2.1.2. REM-suppressive systems: REM-off neurons

The neurons described in the previous section that increase discharge rates with the advent of REM have been termed "REM-on neurons". In contrast, groups of other neurons that radically decrease and may nearly arrest discharge activity with the approach and onset of REM are often termed "REM-off" neurons. The typical discharge activity profile is for discharge rates to be highest in waking, then decrease in synchronized sleep and with near cessation of discharge in REM sleep.

REM-off neurons are distinctive both because they are in the minority in the brain and also because they are recorded in zones with neurons that use biogenic amines as neurotransmitters. The loci include a midline zone of the brainstem raphe nuclei, and a more lateral band-like zone in the rostral pons/midbrain junction that includes the nucleus locus coeruleus, a reticular zone and the peribrachial zone.

2.1.2.1. Raphe nuclei. Neurons with an REM-off discharge profile were first described by McGinty and Harper in the dorsal raphe nucleus (DRN), a finding confirmed by other workers [31–34]. Neurons with the same REM-off discharge pattern have been found in the other raphe nuclei, including nucleus linearis centralis [31,35], centralis superior [36], raphe magnus [37,38], and raphe pallidus [39]. Identification of these extracellularly recorded neurons with serotonin-containing neurons was made on the basis of recording site location in the vicinity of histochemically identified serotonin neurons and the similarity of the extracellularly recorded slow, regular discharge pattern to that of histochemically identified serotonergic neurons *in vitro*. Nonserotonergic neurons in the raphe system have been found to have different discharge pattern characteristics. While this extracellular identification methodology does not approach the "gold standard" of intracellular recording and labeling, the circumstantial evidence that the raphe REM-off neurons are serotonergic appears strong.

2.1.2.2. Locus coeruleus. The second major locus of REM-off neurons is the locus coeruleus, as described in the cat [40,41], rat [42] and monkey [43]. The argument that these extracellularly recorded discharges are from norepinephrine-containing neurons parallels that for the putative serotonergic REM-off neurons. Extracellularly recorded neurons that are putatively noradrenergic have the same slow, regular discharge pattern as norepinephrine-containing neurons identified *in vitro* and have the proper anatomical localization of recording sites, including recording sites in the compact locus coeruleus in the rat, where the norepinephrine-containing neurons are rather discretely localized. Thus, while the evidence that these REM-off neurons are norepinephrine-containing is indirect and circumstantial, it, nonetheless, appears quite strong.

Finally, the remaining groups of REM-off neurons are principally localized to the anterior pontine tegmentum/midbrain junction either in the peribrachial zone, or in a more medial extension of it, recording sites that correspond to the presence of aminergic neurons scattered through this zone. The "stray" REM-off neurons in other reticular locations also correspond to dispersed adrenergic neuronal groups, although adrenergic identification in this case is much less secure. At this point we note that putatively dopaminergic neurons in substantia nigra and midbrain *do not* alter their discharge rate or pattern over the sleep–wake cycle [44], and, thus, are unlikely to play important roles in sleep–wake cycle control.

However, Lu et al. [45] have found dopaminergic neurons in the rat ventral periaqueductal gray (vPAG) that express cFos during wakefulness, fulfilling cFos criteria for wake-active neurons. This population was localized near the DRN and was interspersed with DRN serotonergic neurons at the vPAG level of the dopaminergic neurons. Although the DRN serotonergic neurons are wake-active in unit recordings, they did not express cFOS in the Lu et al. study with exposure to the same degree of wakefulness as the dopaminergic neurons. These dopaminergic neurons projected to cholinergic neurons in the basal forebrain and the LDT, as well as to the monoaminergic cells in the locus coeruleus and DRN and to lateral hypothalamic orexin neurons, and, thus, have projections to zones important in sleep–wake control, as well as to the thalamus and cortex. Unit recordings will be important in confirming the wake-active nature of these dopaminergic neurons, although their admixture with serotonergic neurons will make identification difficult.

2.1.2.3. Do REM-off neurons play a permissive, disinhibitory role in REM sleep genesis by interacting with cholinergic REM-on neurons?. The intriguing reciprocity of the discharge time course of REM-off and REM-on neurons led to the initial hypothesis of interaction between these two groups, as originally proposed for the REM-off adrenergic neurons [40,41,46a,46b]. The phenomenological, behavioral and cellular data have been sufficiently strong so that diverse groups of investigators have proposed that the REM-off neurons, as a complete or partial set, act in a permissive, disinhibitory way on some or all of the components of REM sleep, and we will here summarize these postulates, as well as present some of the data on which they are based. Many of these theories arose as increased technical capability led to extracellular recordings of REM-off neurons.

Dorsal raphe serotonergic neurons. The possibility that the dorsal raphe serotonergic neurons act to suppress one of the major phenomena of REM sleep, PGO waves, was explicitly proposed by Simon et al. [47], on the basis of lesion data, and *in vivo* pharmacological experiments using reserpine [48], which depleted brainstem serotonin and simultaneously produced nearly continuous PGO-like waves. The study of McGinty and Harper [49] was the first of many to document the inverse relationship between the discharge activity of extracellularly recorded dorsal raphe neurons and REM sleep. With respect to REM sleep onset, the decrease in discharge activity of presumptively serotonergic raphe neurons is remarkably consistent. Using a cycle-averaging technique, Lydic et al. [50] found that

the time course of presumptively serotonergic dorsal raphe neuronal activity over the sleep–wake cycle was very clear: waking > NREM > REM sleep. There was also a clear inverse relationship between PGO waves and dorsal raphe discharge, and a premonitory increase in dorsal raphe activity prior to the end of the REM sleep episode, a phenomenon also observed and commented upon by Trulson and Jacobs [34].

Evidence that dorsal raphe serotonergic activity inhibited REM sleep also came from *in vivo* pharmacological experiments [51] and dorsal raphe cooling by Cespuglio et al. [52]. Hobson et al. [41] and McCarley and Hobson [46a] originally proposed that monoaminergic neurons might inhibit REM-on cholinergic REM-promoting neurons, now known to be in LDT/PPT. This postulate of monoaminergic inhibition of cholinergic neurons was originally regarded as extremely controversial. However, interest was quickened by the following series of findings: (1) documentation of serotonergic projections from the dorsal raphe to the mesopontine cholinergic neurons in the (LDT) and pedunculopontine (PPT) nuclei that are implicated in the production of REM sleep [42,53–55]; (2) *in vitro* demonstration of serotonergic inhibition of mesopontine cholinergic neurons [56,57]; (3) the report that microinjection of a serotonergic 5-HT1A agonist into the PPT inhibits REM sleep [58]; and (4) the finding that the level of serotonin release in the cat DRN [59] paralleled the behavioral state ordering at distant DRN projection sites: waking > SWS > REM sleep in both rats [60,61] and cats [62]: waking > SWS > REM sleep, suggesting that this would also be true at axonal release sites in the LDT/PPT.

Since axon collaterals of DRN serotonergic neurons inhibit this same DRN population by way of somatodendritic 5-HT1A receptors [63], it followed that the introduction of a selective 5-HT1A receptor agonist in the DRN by way of microdialysis perfusion should produce strong inhibition of serotonergic neural activity, which would be indicated by a reduction of 5-HT release in the DRN. Moreover, if the hypothesis of serotonergic inhibition of REM-promoting neurons were correct, the inhibition of DRN serotonergic activity should disinhibit REM-promoting neurons, producing an increase in REM sleep concomitant with the changes in DRN extracellular serotonin. Portas et al. [64] tested the effects of microdialysis perfusion of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a selective 5-HT1A receptor agonist, in freely moving cats. In perfusions during waking, DRN perfusion of 8-OH-DPAT decreased 5-HT levels by 50% compared with artificial cerebrospinal fluid (ACSF) (Fig. 6), presumptively through 5-HT1A auto receptor-mediated inhibition of serotonergic neural activity. Concomitantly, the 8-OH-DPAT perfusion produced a short-latency, ~3-fold increase in REM sleep, from a mean of 10.6% baseline to 30.6% ($p < 0.05$, $N = 5$ animals), while waking was not significantly affected (Fig. 3). In contrast, and suggesting DRN specificity, 8-OH-DPAT delivery through a probe in the aqueduct did not increase REM sleep but rather tended to increase waking and decrease SWS.

These data in the cat were confirmed in the rat by Bjorvatn et al. [65] who found that the perfusion of 8-OH-DPAT DRN led to a fourfold increase in REM sleep, while the other vigilance states were not significantly altered. In the cat, Sakai and Crochet [66] failed to replicate the findings of Portas et al. [64] in the cat and Bjorvatn et al. [65] in the rat, perhaps due to technical differences (see [3]). In contrast to the just-described positive findings related to DRN and REM control, Lu et al. [45] found that 5,7-dihydroxytryptamine lesions of the rat serotonergic DRN did not affect REM or other behavioral states.

In vivo and in vitro evidence of serotonergic inhibition of LDT/PPT neurons. The data from Portas et al. [64], however, did not directly demonstrate serotonergic inhibition of neurons in the cholinergic LDT/PPT. Moreover, the presence of some neurons with REM-on and other neurons with wake/REM-on activity in LDT/PPT was a puzzle in terms of the global changes in monoaminergic inhibition. McCarley et al. [67] postulated that while monoamines might inhibit REM-on cholinergic neurons, wake/REM-on neurons might not be inhibited, thus explaining their continued activity in waking; since serotonergic activity is highest during wakefulness, the observed high discharge rate of wake/REM-on neurons during wakefulness would not be consistent with a high level of serotonergic inhibition from a high level of DRN activity. *In vitro* data were also consistent with a subset, not the entire population, of LDT/PPT cholinergic neurons inhibited by serotonin acting at 5-HT1A receptors [56,57]. Thakkar and collaborators [68] developed a novel methodology allowing both extracellular single cell recording and local perfusion of neuropharmacological agents by way of an adjacent microdialysis probe in freely behaving cats to test this hypothesis of differential serotonergic inhibition as an explanation of the different state-related discharge activity. Discharge activity of REM-on neurons was almost completely suppressed by local microdialysis perfusion of the selective 5-HT1A agonist 8-OH-DPAT, while this agonist had minimal or no effect on the wake/REM-on neurons, as illustrated in Fig. 4. Of note, the ordering of 5-HT concentrations in the cholinergic PPT is wake > NREM > REM, consistent with the unit discharge data and, moreover, application of the 5HT1A agonist 8-OH DPAT to the PPT suppressed REM sleep and increased wakefulness ([69], unpublished data).

The finding that only a subpopulation of the recorded LDT/PPT cells were inhibited by 8-OH-DPAT is consistent with rat *in vitro* pontine slice data; intracellular recording and subsequent labeling of the neuron as cho-

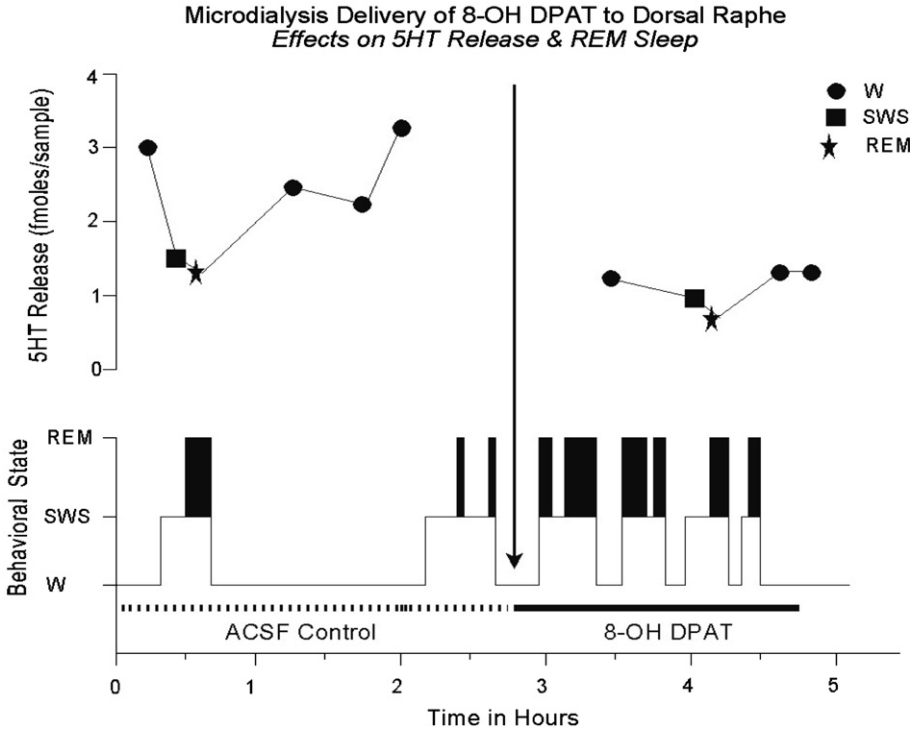

Fig. 3. Time course of 5-HT levels (top portion of figure) and behavioral state (bottom portion of figure) during control DRN ACSF perfusion (interrupted horizontal line) and during DRN 8-OH-DPAT perfusion (solid horizontal line) in a typical experiment. Note that, prior to perfusion, waking DRN 5-HT levels (circles) are higher than those in slow wave sleep (squares) and REM sleep (stars). Each 5-HT value is expressed as fmoles per 7.5 μl sample, and was obtained during an uninterrupted 5-min sequence of the behavioral state. Upon the onset of 10 μM 8-OH-DPAT perfusion (arrow), the 5-HT level dropped quickly to levels as low as those normally present in SWS or REM. Behaviorally, 8-OH-DPAT administration markedly increased REM sleep (black bars in the hypnogram). Adapted from Portas et al. [64].

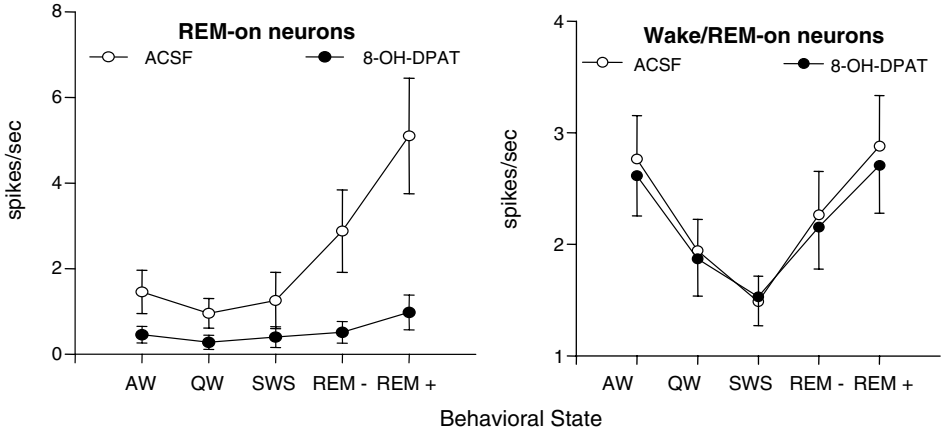

Fig. 4. State-related activity of units in the cholinergic LDT and PPT and the effects of a serotonin 1A agonist applied by microdialysis. Left panel. REM-on units ($N = 9$): Grand mean (+SEM) of discharge rate in each behavioral state before (open circle, ACSF) and after (closed circle) 10 μM 8-OH-DPAT was added to the perfusate. Note suppression of activity (highly statistically significant). Abbreviations are defined in text. Right panel. Wake/REM-on units ($N = 25$): Grand mean (+SEM) of discharge rate of before (open circle, ACSF) and after (closed circle) 10 μM 8-OH-DPAT was added to the perfusate. Note minimal effect of 8-OH-DPAT, not statistically significant. Adapted from Thakkar et al. [68].

linergic found that some, but not all, of the cholinergic neurons in the LDT/PPT were inhibited by serotonin [57]. The different percentages of LDT/PPT neurons that are inhibited by serotonin or serotonin agonists *in vitro* (64%) compared with the [68] *in vivo* findings (36.4%) may be due to anatomical differences between species (rat vs. cat) and/or different concentrations of agents at the receptors.

Locus coeruleus and REM sleep phenomena.

Lesion and cooling studies of locus coeruleus. Lesion studies furnish an unclear picture of the role of the locus coeruleus in REM sleep. Bilateral electrolytic lesions of the locus coeruleus in the cat by Jones et al. [70] led these workers to conclude the locus coeruleus was not necessary for REM sleep. However, following unilateral locus coeruleus electrolytic lesions, Caballero and De Andres [71] found a 50% increase in the percentage of REM sleep ($p < 0.001$) in cats while lesions in neighboring tegmentum and sham-operated controls showed no change. Caballero and DeAndres [71] attributed the differences between their study and that of Jones et al. [70] to nonspecific effects, including urinary retention, of the larger lesions that may have led to a reduction of REM sleep. Cespuglio et al. [72] performed unilateral and bilateral cooling of the locus coeruleus in felines, using the same methodology as for the dorsal raphe cooling. In repeated cooling trials, REM sleep was repetitively induced, and the percentage of REM sleep increased by 120% over control periods. However, Lu et al. [45] found that 6-hydroxydopamine lesions of the rat noradrenergic locus coeruleus did not affect REM sleep or vigilance states.

Site(s) of REM-off and REM-on interaction. The model for REM sleep control proposed here discusses REM-off suppression of REM-on neurons. It must be emphasized that there are several, nonmutually exclusive possible sites of interaction. These include direct noradrenergic-cholinergic (NE–Ach) interactions in the LDT and PPT. For example, there is now evidence that ChAT-labeled fibers are present in the locus coeruleus, and it has long been known that the NE-containing locus coeruleus neurons also stain intensely for the presence of acetylcholinesterase (see review of NE–ACh anatomical interrelationship in [3]). NE varicosities are present throughout the reticular formation and the LDT and the peribrachial area that is the site of ChAT-positive neurons. Thus, NE–ACh interactions may take place directly between these two species of neurons and/or may take place at reticular neurons.

2.1.3. GABAergic influences and REM sleep

In addition to the monoamines and acetylcholine as modulators and controllers of the sleep cycle, there is accumulating and quite strong evidence that GABAergic influences may play an important role. Defining the role of GABA with certainty is difficult, however. Since GABA is a ubiquitous inhibitory neurotransmitter, purely pharmacological experiments using agents that increase or decrease GABA do not answer a key question, namely whether the results so obtained were representative of the increases or decreases in GABA that occur naturally in the course of the sleep cycle, or were simply and trivially the result of a pharmacological manipulation of GABA systems not naturally playing a role in sleep cycle control. Microdialysis is potentially a very useful way of sampling naturally occurring changes in GABA levels over the sleep cycle but is often limited in sensitivity and hence in time resolution of when the changes occur in the sleep cycle.

This section surveys GABA data from dorsal raphe, locus coeruleus, and PRF that are relevant to sleep–wakefulness control. From the standpoint of sleep cycle control, one of the most puzzling aspects has been defining what causes the REM-off neurons in the locus coeruleus and DRN to slow and cease discharge as REM sleep is approached and entered. The reciprocal interaction model (see below) hypothesized a recurrent inhibition of locus coeruleus/DRN might account for this. While recurrent inhibition is present, there is no clear evidence that it might be the causal agent in REM-off neurons turning off. Thus, the prospect that GABAergic mechanism might be involved is of great intrinsic interest.

2.1.3.1. Dorsal raphe nucleus (DRN). Microdialysis in the DRN. Nitz and Siegel [73] reported a significant increase in GABA levels in REM sleep compared with wakefulness while SWS did not significantly differ from wakefulness. Moreover, there was a 67% increase in REM sleep observed with microinjections of the GABA agonist muscimol into the DRN while reverse microdialysis of the GABA antagonist picrotoxin completely abolished REM sleep. For comparative purposes we note that the ~3-fold increase in REM sleep observed with microdialysis application of the 5HT1A agonist 8-OH-DPAT to DRN by [64], and described above, was greater, suggesting that factors other than GABA might influence serotonergic neurons. Although the data did not directly support GABAergic inhibition as a mechanism of the slowing of serotonergic unit discharge in the passage from wakefulness to SWS, Nitz and Siegel noted the possibility of a small increase in the release of GABA, possibly beyond the resolution of the microdialysis technique, might be sufficient to reduce DRN unit discharge in SWS, a suggestion indirectly supported by data from Levine and Jacobs [74].

Microiontophoresis of DRN neurons. Gervasoni et al. [75] reported that, in the unanaesthetized but head-restrained rat, the iontophoretic application of bicuculline on rodent DRN serotonergic neurons, identified by their discharge characteristics, induced a tonic discharge during SWS and REM and an increase of discharge rate during quiet waking. They postulated that an increase of a GABAergic inhibitory tone present during wakefulness was responsible for the decrease of activity of the DRN serotonergic cells during slow wave and REM sleep. In addition, by combining retrograde tracing with cholera toxin B subunit and glutamic acid decarboxylase (GAD) immunohistochemistry, they

provided evidence that the GABAergic innervation of the DRN arose from multiple distant sources and not only from interneurons as classically accepted. Among these afferents, they suggested GABAergic neurons located in the lateral preoptic area and the pontine vPAG, including the DRN itself, could be responsible for the reduction of activity of the DRN serotonergic neurons during SWS and REM sleep, respectively. However, Sakai and Crochet [66], in felines were unable to block the cessation *in vivo* extracellular discharge of presumed serotonergic DRN neurons during REM sleep by either bicuculline or picrotoxin application via a nearby microdialysis probe. While it is entirely possible that GABA pharmacological actions could differ radically in the cat and rat, the most parsimonious interpretation is that the two series of experiments had technical differences.

2.1.3.2. Locus coeruleus. Microdialysis in locus coeruleus. The single published study on sleep–wake analysis of GABA release in the locus coeruleus region [76] found GABA release increased during REM sleep while GABA release during SWS showed a trend-level significance ($p < 0.06$) when compared with waking, while concentrations of glutamate and glycine did not change across sleep and wake states. These data, because of the SWS differences, appear to offer more direct support for locus coeruleus than for DRN neurons for the hypothesis of GABA induced inhibition causing the reduction in locus coeruleus/DRN discharge in SWS and virtual cessation of firing in REM sleep.

Microiontophoresis of locus coeruleus neurons. Gervasoni et al. [77] applied their methodology of microiontophoresis and single-unit extracellular recordings in the locus coeruleus of unanaesthetized, head-restrained rats. Bicuculline, a GABA-A receptor antagonist, was able to restore tonic firing in the locus coeruleus noradrenergic neurons during both REM sleep (in contrast to its effects in the DRN) and SWS. Application of bicuculline during wakefulness increased discharge rate. These data, combined with Nitz and Siegel [76], are, thus, consistent with GABAergic inhibition in the locus coeruleus during REM and SWS.

2.1.3.3. GABA and the PRF: disinhibition and REM sleep. Pharmacological studies in cats on the behavioral state effects of GABA agents. Xi et al. [78,79] have provided pharmacological evidence of GABA suppression of REM-using agents injected into the feline nucleus pontis oralis (NPO), in a region about 2 mm lateral to the midline and more than 1-mm ventral to the locus coeruleus, a region where carbachol induced a short-latency (<4 min) onset of REM sleep. Here, GABA agonists (both A and B) induced wakefulness while antagonists (both A and B) increased REM in felines, suggesting that pontine GABAergic processes acting on both GABA-A and -B receptors might play a critical role in generating and maintaining wakefulness and in controlling the occurrence of the state of REM sleep.

Pharmacological studies in rats on the behavioral state effects of GABA agents. In the head-restrained rat, Boissard et al. [80] used microiontophoresis of the GABA-A antagonists bicuculline and gabazine in the PRF just ventral to the locus coeruleus and LDT, termed the dorsal and alpha subCoeruleus nuclei by Paxinos and Watson [81] and the sublaterodorsal nucleus (SLD) by Swanson [82] (see also Section "PRF ventral to the locus coeruleus"). These agents produced an REM-like state with prominent muscle atonia, but the EEG power spectrum was more similar to waking, with little theta activity and no rapid eye movements or penile erections. In contrast to the cat, carbachol applied to the SLD in these head-restrained rats produced wakefulness and not REM sleep. These data suggested a role of GABA disinhibition in producing some REM-like phenomena, especially muscle atonia.

Sanford et al. [83] assessed REM after bilateral microinjections into the rostral PRF of muscimol (suppressed REM) and bicuculline (enhanced REM) in rats during the light (inactive) period, but they did not observe the pronounced short-latency, long-duration increase in REM seen in cats [79]. Repeating these experiments in the dark (active) phase would help determine whether the strongly circadian rat differs from the cat as a function of circadian phase.

Microdialysis measurements of GABA in the feline PRF. Recently Thakkar et al. ([84], unpublished data) have studied GABA release in the PRF of freely moving cats, after validating GABA measurements by pharmacologically increasing/decreasing GABA release. In the four PRF sites thus far tested, multiple episodes of REM sleep had consistently lower levels of GABA than wakefulness. Although wake was not statistically different from SWS, there was a trend toward lower GABA levels in SWS. These data provide very preliminary but direct evidence compatible with GABA disinhibition in the PRF during REM sleep.

2.2. A model of REM sleep generation incorporating GABAergic neurons

We here briefly summarize a structural model of REM sleep cyclicity, based on the data discussed above, and note that Steriade and McCarley [1] have a much more complete exposition. The history of the development of structural models encompasses the history of discovery of neurons and neurotransmitters important in REM sleep and is one of ever-growing complexity. The first formal structural and mathematical model was presented in 1975 by McCarley and Hobson [46a]. This model, termed the Reciprocal Interaction model, was based on the interaction of populations of

REM-on and REM-off neurons and mathematically described by the Lotka–Volterra equations, derived from population models of prey–predator interaction. We suggest that the basic notion of interaction of REM-on and REM-off neuronal populations is a very useful one for modeling and conceptualization, even though the description of the populations of neurons characterized as REM-on and REM-off has been altered and made much more detailed. Fig. 5 describes the "core" features of the structural and mathematical model, namely the interaction of REM-on and REM-off neurons, and provides a description of the dynamics.

Fig. 6 identifies the neurotransmitter components of the REM-on and REM-off interaction described in Fig. 5. Steriade and McCarley [1] provide a detailed account of the evidence supporting the model. In this model, cholinergic neurons promote REM through action on reticular effector neurons, which also provide positive feedback onto the cholinergic neurons, LDT/PPT → PRF → LDT/PPT. (Mathematically, this is the basis of the postulate of self-excitation (positive feedback) and exponential growth of REM-on neurons, term "a" in Fig. 5).

2.2.1. Reticular formation and GABAergic influences

Not only may LDT/PPT cholinergic input excite PRF neurons, but there is the intriguing possibility that inhibitory LDT/PPT projections from REM-on neurons impinge onto GABAergic PRF interneurons with projections onto PRF neurons. This would have the effect of disinhibiting glutamatergic PRF neurons as REM sleep was approached and entered. Gerber et al. [85] found that about one-fourth of PRF neurons *in vitro* were inhibited by muscarinic cholinergic agents. Whether these neurons that were inhibited were GABAergic or not, however, is still not known. Preliminary data in the cat support cholinergic inhibition of GABAergic neurons, since microdialysis application of carbachol to the PRF not only induced REM but decreased GABA concentrations in samples from the same microdialysis probe [84]. Moreover, as outlined above, there is considerable evidence that reduction of GABA inhibition in the PRF might play a role in production of REM sleep. First, there are preliminary microdialysis data in both the cat [84] and the rat [86] that GABA levels in the PRF are decreased during REM sleep compared to wakefulness, and the Thakkar et al. [84] data indicate that levels in NREM sleep are intermediate between wakefulness and REM sleep. Second, pharmacological experiments support this concept since GABA antagonists applied to the rostral PRF-produced REM sleep in both cat [78,79] and rat [83]. This postulated pathway of LDT/PPT muscarinic inhibition of GABA PRF neurons during REM sleep is illustrated in Fig. 6. The dotted lines for this and other GABAergic pathways indicate the more tentative nature of identification of both the projections and their source. This figure graphically emphasizes that that inhibition of PRF GABAergic neurons that inhibit PRF neurons would "disinhibit" the PRF neurons and so constitute an

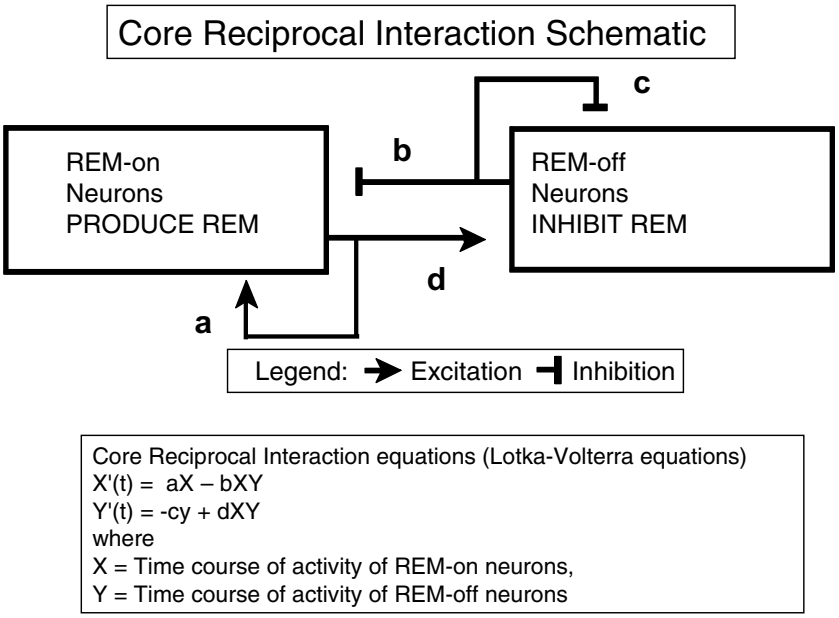

Fig. 5. Summary of the "core" features of the reciprocal interaction model. The REM-on neuronal population has positive feedback so that activity grows (see connection labeled "a"). This activity gradually excites the REM-off population (connection "d"). The REM-off population then inhibits the REM-on population (connection "b"), terminating the REM episode. The REM-off population is also self-inhibiting (connection "c"), and as REM-off activity wanes, the REM-on population is released from inhibition and is free to augment its activity. This begins a new cycle of events. This interaction is formally described by the Lotka–Volterra equations, where X = REM-on activity and Y = REM-off activity.

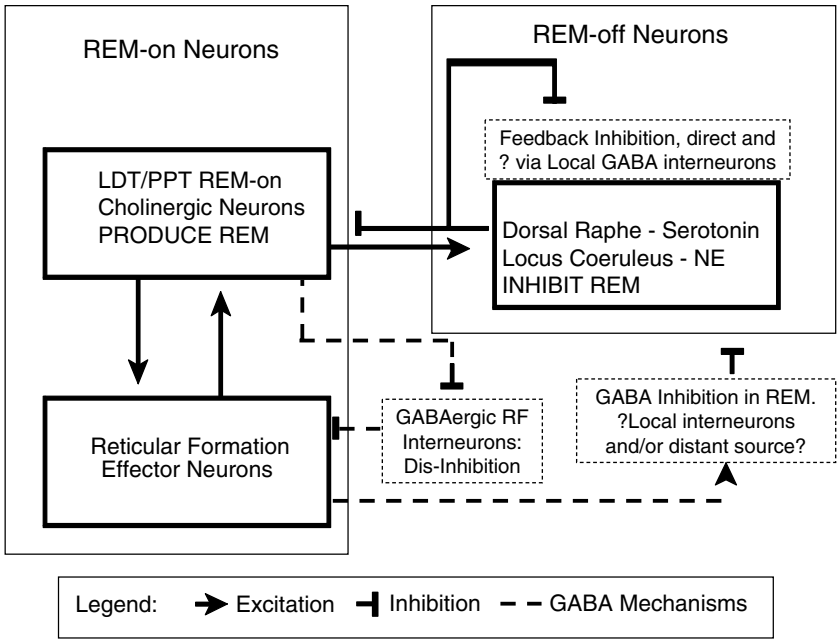

Fig. 6. A structural model of REM sleep control. See text for description.

additional source of positive feedback. Of note, the GABA levels in wake and in REM in the PRF [84] described above are almost the exact inverse of the measurements from Nitz and Siegel [76] of GABA in the locus coeruleus, suggesting a possible common source in the REM on neuronal activity of disinhibition in the PRF and inhibition in the locus coeruleus REM-on neurons (PRF wake/REM ratio = 1.7 and locus coeruleus REM/wake ratio = 1.7; also see GABA discussion above).

2.2.2. REM-off neurons and their excitation by REM-on neurons. (Fig. 5 term "d")

There is anatomical evidence for cholinergic projections to both the locus coeruleus and DRN [87]. *In vitro* data indicate excitatory effects of acetylcholine (ACh) on locus coeruleus neurons, but data do not support such direct effects on the dorsal raphe [88]. The REM-on neuronal excitation of dorsal raphe neurons may be mediated through the reticular formation; there is *in vitro* evidence for glutamatergic excitatory effects on both locus coeruleus and dorsal raphe neurons.

2.2.3. Inhibition of REM-on neurons by REM-off neurons (Fig. 5 term "b")

For many years, this aspect of the model was most controversial, since the indirect evidence from *in vivo* data, although generally supportive, was subject to alternative explanations. Now *in vitro* data indicate a subpopulation of cholinergic neurons in the LDT are inhibited by serotonin [57]. Inhibition is especially consistent for the population of LDT neurons that fire in bursts; such burst firing has been shown by *in vivo* extracellular recordings to be tightly correlated with lateral geniculate nucleus PGO waves, which other data indicate are cholinergically mediated. The action potential burst itself is caused by a particular calcium current, the low threshold spike (LTS), which causes calcium influx and depolarization to a level that produces a burst of sodium-dependent action potentials. Some nonburst cholinergic neurons are also hyperpolarized by serotonin. Other data indicate that the effects of norepinephrine (NE) on LDT/PPT cholinergic neurons are also inhibitory [89]. Moreover, noncholinergic, presumptively GABAergic interneurons, are excited by NE [90]; GABAergic interneurons acting to inhibit cholinergic neurons would furnish yet another possible mechanism of inhibition of cholinergic mesopontine neurons by NE, thus further strengthening the model's postulates.

2.2.4. Inhibitory feedback of REM-off neurons (Fig. 5 term "c")

There is strong *in vitro* physiological evidence for NE inhibition of locus coeruleus neurons and of serotonergic inhibition of dorsal raphe neurons, and anatomical studies indicate the presence of recurrent inhibitory collaterals. However, there is no clear evidence that these recurrent collaterals are responsible for REM-off neurons turning off as REM sleep is approached and entered. Indeed, from the standpoint of sleep cycle control, one of the most puzzling aspects has been defining what causes the "REM-off" neurons in the locus coeruleus and DRN to slow and cease discharge as REM sleep is approached and entered. Thus, the prospect that a GABAergic mechanism might be involved is of great intrinsic interest. As reviewed above, supporting a GABAergic mechanism in the DRN is the *in vivo*

microdialysis finding by Nitz and Siegel [73] that in naturally sleeping cats there is a significant increase in DRN GABA levels in REM sleep. Moreover, as discussed above, the balance of pharmacological studies support a GABA-induced suppression of DRN activity. We think it is important to emphasize that the issue of GABAergic and serotonergic inhibition as important in suppression of DRN discharge is not an either/or issue but rather is likely one of joint influences, since, as noted above, the ~3-fold increase in REM sleep observed with microdialysis application of the 5HT1A agonist 8-OH-DPAT to DRN by Portas et al. [64] was greater than that observed with the GABA agonist muscimol by Nitz and Siegel [73], suggesting that factors other than GABA might influence serotonergic neurons. Determination of whether the GABA time course of release parallels the decrease in activity of DRN serotonergic neurons during SWS as REM is approached awaits better technology for measurement of GABA with short duration collection periods. GABAergic influences in the locus coeruleus during REM sleep have been described above in the microdialysis experiments of Nitz and Siegel [76] and the microiontophoresis studies of Gervasoni et al. [77].

2.2.5. Source of GABAergic inputs to the locus coeruleus and DRN

Overall, the DRN and LC findings of increased GABA during REM are consistent with but do not prove the hypothesis that increased GABAergic inhibition leads to REM-off cells turning off. The increased GABAergic tone could simply be a *consequence* of other state-related changes without causing these changes. A major missing piece of evidence on GABAergic inhibition of LC/DRN and REM-off neurons is the recording of GABAergic neurons whose activity has the proper inverse time course to that of LC and DRN neurons (see review in [1]). In our diagram in Fig. 6 of the brainstem anatomy of REM sleep cycle control, we have suggested that GABAergic neurons in the PRF might provide the input to DRN/LC. Certainly neurons in the PRF have the requisite time course of activity, but there is, to date, no evidence that these are GABAergic neurons. Within the LC and DRN, Maloney et al. [24] found the extent of cFos labeling of GABAergic (GAD-positive) neurons in DRN and LC to be inversely correlated with REM sleep percentage, and to decrease in recovery from REM sleep deprivation. This is, of course, compatible with a local source of GABA increase during REM. However, unit recordings in DRN and LC have not found evidence for neurons with an inverse time course to that of the presumptively monoaminergic LC and DRN neurons, suggesting no local source of GABA input. We, thus, survey data about other sites of GABAergic input as to where these neurons might be located.

2.2.5.1. Periaqueductal Gray (PAG).

The Gervasoni et al. [75] study on DRN pointed to the PAG as a possible source of the GABAergic input proposed to inhibit DRN neurons. In accord with this hypothesis, both vlPAG lesions [91] and muscimol injections [92] produced a large increase in REM sleep. Thakkar and colleagues [93] recorded vlPAG unit activity in freely behaving cats, but none of the 33 neurons showed a tonic discharge increase before and during REM, but rather were phasic in pattern and increased discharge rate too late in the cycle to be a cause of the DRN SWS suppression. These data, thus, suggest that, although vlPAG neurons may regulate phasic components of REM sleep, they do not have the requisite tonic pre-REM and REM activity to be a source of GABAergic tone to monoaminergic neurons responsible for their REM-off discharge pattern. The negative findings would suggest that, at a minimum, neurons with the requisite activity are not abundant in the vlPAG.

2.2.5.2. Ventro-lateral preoptic area (VLPO).

This forebrain site was retrogradely labeled by Gervasoni et al. [75] as projecting to the DRN. Forebrain influences on REM sleep are discussed in the next section, but the Jouvet transection experiments suggest, however, these are not essential for the basic REM cyclicity found in the pontine cat. Another section of this paper discusses VLPO influences on the brainstem.

2.2.5.3. GABAergic neurons in the subCoeruleus (SubC), pontine nucleus oralis (PnO), and lateral pontine tegmentum (LPT)?.

Recent preliminary data from mice with a Green Fluorescent Protein Knock-in under control of the GAD67 promoter point to these locations as possible sites (see [94]). These mice have GABAergic neurons that are identifiable during the recording session in the *in vitro* slice by their fluorescence. In all of these locations a subset of GABAergic neurons was found that was excited by the acetylcholine receptor agonist Carbachol, and, thus, the cholinergic activity prior to and during REM sleep would excite these GABAergic neurons, and, thus, inhibit target neurons in the LC and DRN. Lu et al. [45] have reported GABAergic neurons in the LPT that express cFos during REM and are REM-on by cFos criteria, although their action potential activity has not been recorded.

2.2.6. An alternative REM-on and REM-off model with GABAergic neurons

Very recently Lu et al. [45] have proposed a GABAergic organization of REM-off and REM-on neurons, based on cFos expression data, lesions and anatomical connectivity mapping, but with no cellular electrophysiological data. (This study notes that their characterization of REM-on and REM-off neuronal activity with cFos must be confirmed by electrophysiological record-

ings, also needed to determine the time course of activity). They find that REM-off (by cFos criteria) GABAergic neurons are present in an arc of brainstem extending from the ventrolateral periaqueductal gray matter (vlPAG) and continuing laterally and ventrally in a reticular area they term the LPT. They suggest that these GABAergic REM-off neurons inhibit REM-on (cFos criteria) GABAergic neurons in what they term, following Luppi, the SLD (equivalent to the subCoeruleus area or perilocus coeruleus alpha in cats) and a dorsal extension of this region, termed the precoeruleus. In turn, the SLD GABAergic REM-on neurons may inhibit GABAergic REM-off neurons in the vlPAG–LPT, suggesting a flip-flop switch arrangement in which each side inhibits the other.

Lu et al. [45] also report evidence that other neurons in this circuit are important in muscle atonia and hippocampal theta. In particular, they found that glutamatergic ventral SLD neurons have direct projections to spinal cord interneurons – apparently not requiring a relay in the medial medulla – that might inhibit spinal motoneurons. Lesions of the vSLD caused episodes of REM sleep without atonia, while animals with lesions of the ventromedial medulla with orexin B–saporin had normal REM atonia. In terms of EEG phenomena of REM sleep, a group of glutamatergic precoeruleus neurons was found to project to medial septum, and lesions of this region abolished REM hippocampal theta. This paper provides a wealth of new data, but Lu et al. [45] do not address how REM sleep periodicity might come about in this flip-flop model. Indeed, from a formal mathematical point of view, two mutually inhibitory populations will not cycle, and some external input would be required for them to get out of a state in which one inhibitory population predominates (the ecological analogy would be two populations of predators, where one would eventually devour the other, rather than the cycling observed in the prey–predator equations of the Lotka–Volterra model). Moreover, the time course of pre-REM neuronal activity in the brainstem is not an immediate transition from SWS to REM, but rather a gradual change (see [1,46a]).

2.3. Orexin/hypocretin effects and modeling circadian control of REM sleep

Another paper in this issue treats orexin/hypocretin, and hence only the circadian control effects on REM are briefly summarized here. Mathematically, a limit cycle model best describes the dynamics of the REM cycle, which retains its basic cyclicity no matter how it is set into motion (for discussion, see [95–98]). The other important feature not addressed in the simple model is circadian variation. Fig. 1 sketches the modeling of the normal course of a night of REM activity in entrained humans. This smaller amplitude and shorter initial first cycle, as well as the absence of REM activity during the day is modeled by having the REM oscillator shut off and modulated by excitatory input to the REM-off neurons. When this excitatory input to the REM-off neurons was not present, this allowed the REM oscillator to become active [96–98]. One of the exciting possibilities is that orexin could be this factor (or one of the factors) exciting the REM-off neurons, consistent with its effects on LC and DRN neurons. Experiments in which the orexin ligand is either knocked down or orexin neurons are destroyed will be useful in determining if these manipulations destroy the circadian modulation of REM sleep, as would be predicted by this hypothesis. The breakthrough of REM-like phenomena during the day in narcolepsy, a disorder characterized by a loss of orexinergic neurons, would be consistent with this hypothesis (see review of orexin and narcolepsy in this issue).

Supporting this possibility are data from transgenic mice and rats in which orexin-containing neurons are destroyed post-natally by orexinergic-specific expression of a truncated Machado-Joseph disease gene product (ataxin-3) with an expanded polyglutamine stretch under control of the human prepro-orexin promoter; this has provided a valuable animal model of narcolepsy [99]. This transgenic rat, compared with wild types, showed a markedly different REM sleep profile. Perhaps the most striking change in REM percentage was the difference in diurnal distribution. REM sleep (including sleep-onset REM (SOREM)) was approximately a two-fold increased over the wild type in the normally REM-poor dark period. Finally, very recent data from Chen et al. [100] indicate that small interfering RNA-induced knockdown of pre-proorexin led to an increase in REM sleep in the active (dark) period of the rat, but not in the light period, as predicted by the circadian REM regulation hypothesis of orexin actions. The mechanisms through which orexin acts in the brainstem is a topic for active investigation. Preliminary work indicates that orexin excites many types of neurons, GABAergic, aminergic and glutamatergic; interaction with other neurotransmitters will likely be important in determining effects on the target neurons [101].

3. NREM sleep and adenosine

This section focuses on nonrapid eye movement (NREM) sleep and adenosine, with a special focus on the basal forebrain. Another chapter in this volume discusses hypothalamic sleep mechanisms.

3.1. EEG Activation

Although it is often termed electroencephalographic (EEG) desynchronization, EEG activation is preferable because it is the EEG pattern accompanying cortical

activity and because higher frequency rhythmic activity (gamma activity, about 40 Hz and higher) may be present, although the amplitude of the higher frequency amplitude is low. The early concept of the "ascending reticular activating system" (ARAS) has given way to the concept of multiple systems important in maintaining wakefulness and an activated EEG (see review in [1]). Systems utilizing the neurotransmitters acetylcholine, norepinephrine, serotonin, and histamine are also important, in addition to the brainstem reticular systems and, the region emphasized in this section, the basal forebrain. The cholinergic system is likely important in activation and, as discussed earlier, we now know that a subset of the cholinergic laterodorsal tegmental nucleus (LDT)/pedunculopontine tegmental nucleus (PPT) neurons has high discharge rates in waking and rapid eye movement (REM) sleep and low discharge rates in slow wave sleep (SWS); this group is anatomically interspersed with the physiologically distinct REM-selective cholinergic neurons. There is also extensive anatomical evidence that these cholinergic neurons project to thalamic nuclei important in EEG activation. In addition to brainstem cholinergic systems, cholinergic input to the cortex from the basal forebrain cholinergic nucleus basalis of Meynert is also important for EEG activation, as are GABAergic and glutamatergic cortical projections from the basal forebrain (see Fig. 7a). Many neurons in this zone are active in both wake and REM sleep and both lesion and pharmacological data suggest their importance in REM sleep (see review in [102]). This basal forebrain cholinergic zone is discussed below in the context of adenosine.

3.2. Adenosine as a mediator of the sleepiness following prolonged wakefulness (homeostatic control of sleep)

A growing body of evidence supports the role of purine nucleoside adenosine as a mediator of the sleepiness following prolonged wakefulness, a role in which its inhibitory actions on the basal forebrain wakefulness-promoting neurons may be especially important. Common-sense evidence for an adenosine role in sleepiness

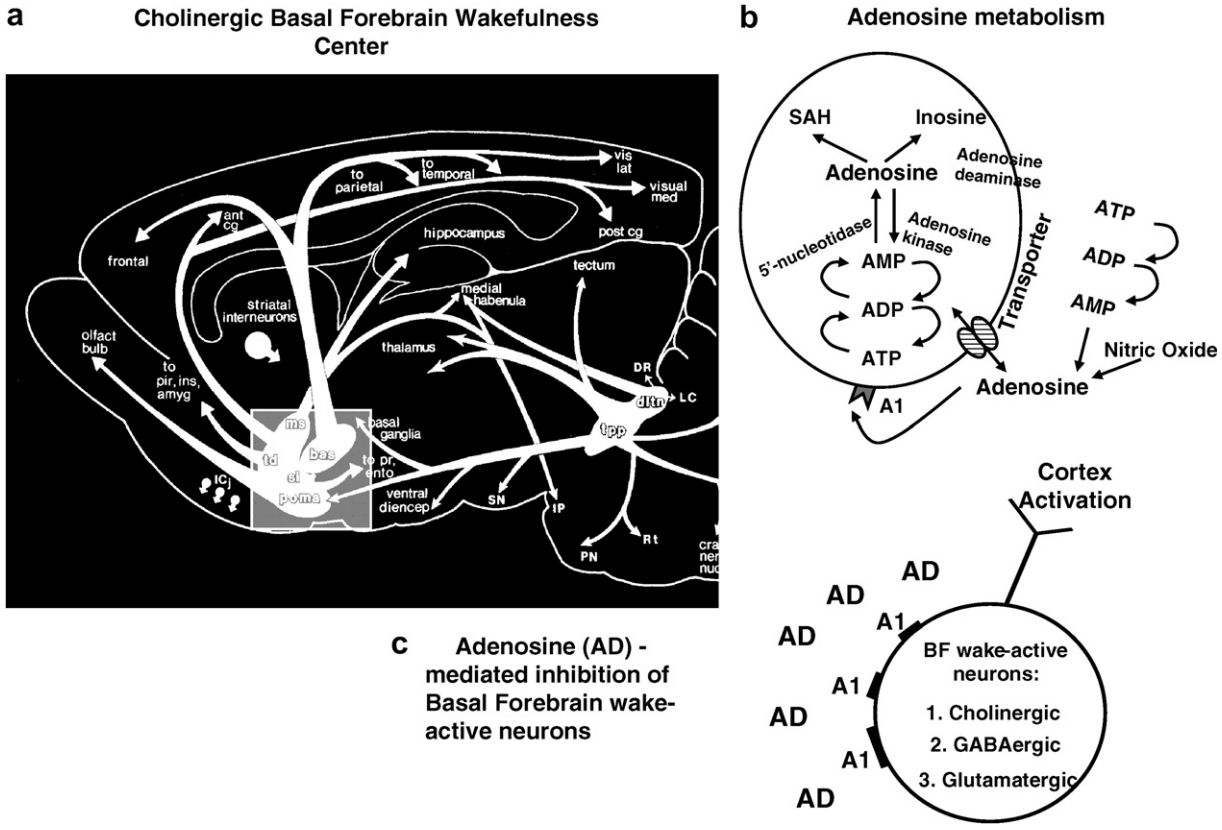

Fig. 7. (a) Cholinergic basal forebrain. (b) Schematic of main intra- and extracellular metabolic pathways of adenosine. The intracellular pathway from ATP (adenosine 5′-triphosphate) to ADP (adenosine diphosphate) to AMP (adenosine monophosphate) to adenosine is respectively regulated by the enzymes ATP-ase, ADP-ase and 5′-nucleotidase and extracellularly by the respective ecto-enzymes. Adenosine kinase converts adenosine to AMP, while adenosine deaminase converts adenosine to inosine. The third enzyme to metabolize adenosine is *S*-adenosylhomocysteine hydrolase, which converts adenosine to *S*-adenosylhomocysteine (SAH). Adenosine concentration between the intra- and extracellular spaces is equilibrated by nucleoside transporters. (c) Schematic of adenosine effects on cells in the basal forebrain. Extracellular adenosine (AD) acts on the A1 adenosine receptor subtype to inhibit neurons of various neurotransmitter phenotypes that promote EEG activation and wakefulness. Modified from McCarley [202].

comes from the nearly universal use of coffee and tea to increase alertness, since these beverages contain the adenosine receptor antagonists caffeine and theophylline (reviewed in [103]). Basheer and co-workers [4] have advanced the hypothesis that, during prolonged wakefulness, adenosine accumulates selectively in the basal forebrain and promotes the transition from wakefulness to SWS by inhibiting cholinergic and noncholinergic wakefulness-promoting basal forebrain neurons via the adenosine A1 receptor.

Adenosine, a ubiquitous nucleoside, serves as a building block of nucleic acids and energy storage molecules, as a substrate for multiple enzymes, and, most importantly for this review, as an extracellular modulator of cellular activity [104a]. Since its first description in 1929 by Drury and Szent-Gyorgyi [104b], adenosine has been widely investigated in different tissues. The endogenous release of adenosine exerts powerful effects in a wide range of organ systems [105]. For example, adenosine has a predominantly hyperpolarizing effect on the membrane potential of excitable cells, producing inhibition in smooth muscle cells both in the myocardium and coronary arteries, as well as in neurons in brain. From an evolutionary point of view, adenosine's postulated promotion of sleep following activity could be considered as an extension of its systemic role in protecting against overactivity, as seen most clearly in the heart.

Adenosine in the central nervous system functions both as a neuromodulator and as a neuroprotector. The modulatory function, reviewed as early as 1981 by Phillis and Wu [106], is exerted under physiological conditions both as a homeostatic modulator as well as a modulator at the synaptic level [107–109]. Adenosine has also been implicated in neuroprotective responses to injury or hypoxia, reducing excitatory amino acid release and/or Ca^{2+} influx, as well as by reducing cellular activity and hence metabolism [110]. Adenosine also has been implicated in locomotion, analgesia, chronic drug use, and mediation of the effects of ethanol, topics which are reviewed in Dunwiddie and Masino [111].

Initial evidence that adenosine, a purine nucleoside, was a sleep factor came from pharmacological studies describing the sleep-inducing effects of systemic or intracerebral injections of adenosine and adenosine-agonist drugs ([112]; reviewed in [113]).The hypnogenic effects of adenosine were first described in cats by Feldberg and Sherwood in 1954 [114] and later in dogs by Haulica et al. in 1973 [115]. Since then, the sedative, sleep-inducing effects of systemic and central administrations of adenosine have been repeatedly demonstrated (e.g., [112,113,116–118]). These effects and the fact that adenosine is a byproduct of energy metabolism, led to postulates that adenosine may serve as a homeostatic regulator of energy in the brain during sleep, since energy restoration has been proposed as one of the functions of sleep [119,120]. Fig. 7b schematizes adenosine metabolism and its relationship to adenosine triphosphate (ATP).

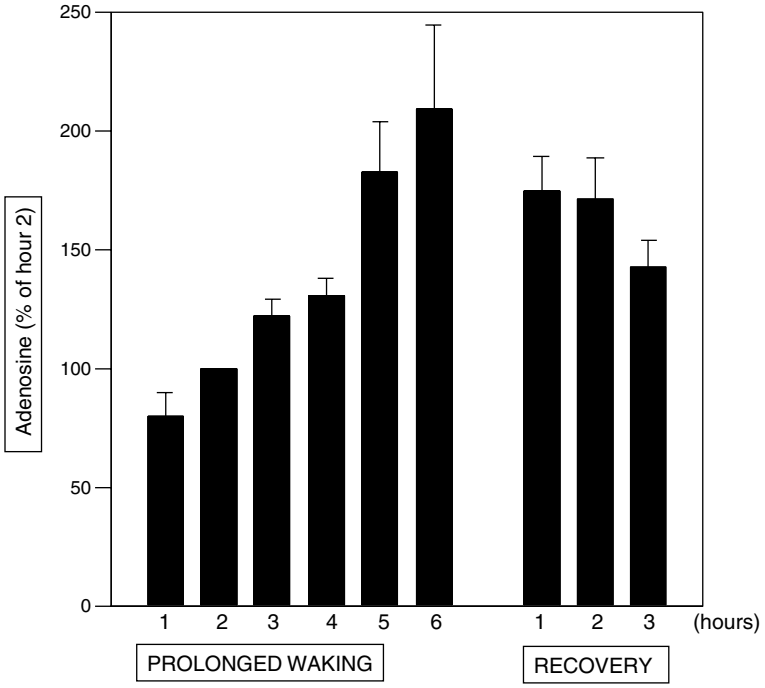

Fig. 8. Mean basal forebrain extracellular adenosine values by hour during 6 h of prolonged wakefulness and in the subsequent 3 h of spontaneous recovery sleep. Microdialysis values in the six cats are normalized relative to the second hour of wakefulness. Adapted from Porkka-Heiskanen et al. [122].

Reasoning that adenosine control of sleepiness might best be understood as an inhibition of wakefulness-promoting neuronal activity, Portas et al. [121] used microdialysis to apply adenosine to the cholinergic neuronal zones of the feline basal forebrain and LDT/PPT, known to be important in the production of wakefulness (see above). At both sites, adenosine produced a decrease in wakefulness and in the activated EEG. (Fig. 7c provides a schematic of this wakefulness-suppressing action in basal forebrain.)

However, these were pharmacological experiments, and the remaining critical piece of evidence was a study of the changes in extracellular concentration of adenosine as sleep–wake state was varied. Using cats to take advantage of the predominance of homeostatic versus circadian control of sleep, Porkka-Heiskanen et al. [122] found extracellular adenosine in the basal forebrain were higher during spontaneously occurring episodes of wake compared with SWS. Moreover, adenosine concentrations progressively increased with each succeeding hour of wakefulness during atraumatic sleep deprivation (Fig. 8).

These investigators also perfused the adenosine transport inhibitor *S*-(4-nitrobenzyl)-6-thioinosine (NBTI, 1 mM) to produce a twofold increase in extracellular adenosine in the basal forebrain, about the same as prolonged wakefulness. Both prolonged wakefulness and NBTI infusion in the basal forebrain produced the same pattern of sleep–wakefulness changes, with a reduction in wakefulness and an increase in SWS, as well as an increase in delta band and a decrease in gamma band power. In contrast, in the ventroanterior/ventrolateral (VA/VL) thalamus, a relay nucleus without the widespread cortical projections of the basal forebrain, increasing adenosine concentrations two-fold in with NBTI had no effect on sleep–wakefulness.

3.3. Site specificity and sources of adenosine increases with prolonged wakefulness

Site specificity of adenosine increases with prolonged wakefulness. A systematic study [123] in multiple brain areas showed that sustained and monotonic increases in adenosine concentrations in the course of prolonged wakefulness (6 h) occurred only in the cat basal forebrain, and to a lesser extent in cerebral cortex. Of note, adenosine concentrations did not increase elsewhere during prolonged wakefulness even in regions known to be important in behavioral state control, such as the preoptic-anterior hypothalamus region (POAH), dorsal raphe nucleus (DRN), and PPT; nor did it increase in the ventrolateral/ventroanterior thalamic nuclei, although adenosine concentrations were higher in all brain sites sampled during the naturally occurring (and shorter duration) episodes of wakefulness as compared to sleep episodes in the freely moving and behaving animals. Not all brain sites were surveyed and so it is possible that some other site(s) might show the same pattern as the basal forebrain. For example, diurnal variations in adenosine concentrations have been found in the hippocampus, although lack of sleep state recording in this study makes it difficult to know if these are primarily related to sleep–wake state or circadian rhythm [124]. It is also important to note that only six hours of prolonged wakefulness were studied and some preliminary data (Basheer et al., unpublished data) suggests more widespread changes with long durations of wakefulness.

These data suggest the presence of brain region-specific differences in factors controlling extracellular adenosine concentration. There are several potential factors controlling the concentration of extracellular adenosine.

(1) *Metabolism.* First, data suggest that the level of extracellular concentration of adenosine is dependent on metabolism, with increased metabolism leading to reduced high energy phosphate stores and increased adenosine which, by way of an equilibrative nucleoside transporter, might lead to increases in extracellular adenosine (see Fig. 7b). For example, in the *in vitro* hippocampus, extracellular adenosine release, shown by ATP labeling with [3H] adenine to be secondary to ATP breakdown, was induced both by hypoxia/hypoglycemia and by electrical field stimulation (Fredholm et al. [125]). Thus, when the energy expenditure exceeded energy production, adenosine levels increased in the extracellular space. Of note also, pharmacologically induced local energy depletion in the basal forebrain, but not in adjacent brain areas, induces sleep [126]. It is worth emphasizing at this point that the equilibrative transporter for adenosine is a nucleoside transporter, and *in vitro* data [125] suggest that the transporter inhibitor NBTI has the effect of increasing adenosine release from the cell and decreasing inosine and hypoxanthine release, in agreement with the *in vivo* measurements of the effects of NBTI on adenosine [122]. Support for an adenosine-metabolism link hypothesis comes from the facts that EEG arousal is known to diminish as a function of the duration of prior wakefulness and also with brain hyperthermia, both associated with increased brain metabolism. Borbely [127] and Feinberg et al., [128] report the effect of wakefulness on reducing EEG arousal. Brain metabolism during delta SWS is considerably less than in wakefulness. In humans, a 44% reduction in the cerebral metabolic rate (CMR) of glucose during delta wave sleep, compared with that during wakefulness, was determined by Maquet et al. [129], and a 25% reduction in the CMR of O_2 was determined by Madsen et al. [130]. Horne [131] has reviewed metabolism and hyperthermia.

(2) ATP release. Another potential factor in the increase in extracellular adenosine during wakefulness is the dephosphorylation of ATP, released as a co-transmitter during synaptic activity by ectonucleotidases.

(3) Regulatory enzymes. The biochemistry of enzymes responsible for adenosine production, as well as its conversion to inosine or phosphorylation to adenosine mono-phosphate (AMP), has been well characterized. In view of the observed selective increase in the levels of extracellular adenosine in the cholinergic basal forebrain with prolonged waking, changes in the activity of regulatory enzymes have been examined following three and 6 h of sleep deprivation in the rat. None of the enzymes in the basal forebrain, including adenosine kinase, adenosine deaminase and both ecto- and endo-5′-nucleotidases, showed any change in activity following sleep deprivation [132,133].

(4) Nucleoside transporters. It is possible that adenosine concentration increases and the regional selectivity might be related to differences in activity of the nucleoside transporters in the membrane, although the lack of knowledge about these transporters and their regulation has hindered sleep-related research. The human (h) and rat (r) equilibrative (Na(+)-independent) nucleoside transporters (ENTs) hENT1, rENT1, hENT2, and rENT2 belong to a family of integral membrane proteins with 11 transmembrane domains and are distinguished functionally by differences in sensitivity to inhibition by NBTI; ENT1 but not ENT2 has pharmacological antagonists, such as NBTI [134,135]. Very little is known about the active transporter. After 6 h of sleep deprivation in the rat, NBTI binding to the ENT1 transporter, a possible indirect measure of ENT1 activity, was found to be decreased in the basal forebrain but not in the cortex, although ENT1 mRNA did not change [136]. Recent preliminary data from Vijay, McCarley, and Basheer (unpublished data) indicate that ENT1 null mice have decreased delta activity both during spontaneous sleep and sleep following deprivation, consistent with data indicating that these mice have reduced adenosine tone.

(5) Nitric oxide (NO). Another candidate for contributing to the increased adenosine concentration following prolonged wakefulness is the release of NO, as demonstrated in hippocampal slices [137] and forebrain neuronal cultures [138]. Infusion of the NO donor diethylamine-NONOate into the cholinergic basal forebrain has been shown to mimic the effects of sleep deprivation by increasing NREM sleep [126]. Recent studies by Kalinchuk and collaborators [139,140] implicate immune NO synthase (iNOS) in the production of NO with prolonged wakefulness and in mediating the increased extracellular adenosine.

Thus, the mechanism for sleep deprivation-induced increase in extracellular adenosine that is specific to the basal forebrain is not yet clear, but transporter differences and NO release are, in the author's opinion, excellent candidates. Of note, the observed differences in adenosine accumulation during wakefulness suggest the mechanisms responsible for these differences, such as differences in transporters or receptors might be targets for pharmaceutical agents and a rational hypnotic.

3.4. Neurophysiological mechanisms of adenosine effects

Arrigoni et al. [141] used whole-cell patch-clamp recordings in *in vitro* brain slices to investigate the effect of adenosine on identified cholinergic and noncholinergic neurons in the magnocellular preptic (MCPO) and substantia innomina regions of the basal forebrain. Adenosine reduced the MCPO/SI cholinergic neuronal firing rate by activating an inwardly rectifying potassium current (I_{Kir}); application of the A1 receptor antagonist 8-cyclo-pentyl-theophylline (CPT) blocked the effects of adenosine. Adenosine was also tested on two groups of electrophysiologically distinct, noncholinergic basal forebrain neurons. In the first group, presumptively GABAergic, adenosine, by activation of postsynaptic A1 receptors, reduced spontaneous firing by inhibition of the hyperpolarization-activated cation current (Ih). Blocking the H-current with ZD7288 (20μM) abolished adenosine effects on these neurons. The second group was not affected by adenosine, and might be identified with sleep–active neurons. Of note, LDT/PPT cholinergic neurons were also found by Rainnie and co-workers [142] to be under the tonic inhibitory control of endogenous adenosine, an inhibition mediated by both I_{Kir} and Ih.

3.5. Receptor mediation of adenosine effects: A1 and A2a subtypes

To date, four different adenosine receptors (A1, A2, A2B, A3) have been cloned in a variety of species, including man [111,143]. All of the adenosine receptors are seven transmembrane domain, G-protein-coupled receptors, and they are linked to a variety of transduction mechanisms. The A1 receptor has the highest abundance in the brain and is coupled with activation of K+ channels (primarily postsynaptically) and inhibition of Ca^{2+} channels (primarily presynaptically), both of which would inhibit neuronal activity (see review in [144]). The A2 receptor is expressed at high levels in only a few regions of the brain, such as the striatum, nucleus accumbens, and olfactory bulb, and is primarily linked to activation of adenylyl cyclase. Evidence is available for both A1 and A2 adenosine receptor subtypes in mediating the sleep-inducing effects of adenosine.

3.5.1. Receptor mediation of adenosine effects: the A1 subtype

A1 receptor. IP or ICV administration of the highly selective A1 receptor agonist, N6-cyclopentyladenosine

(CHA) was found to result in an increased propensity to sleep and increased delta waves during sleep, suggesting a role of the A1 adenosine receptor [145,146]. Studies in the cat and in the rat revealed that the somnogenic effects of adenosine in the cholinergic region of the basal forebrain appear to be mediated by the A1 adenosine receptor, since the unilateral infusion of the A1 receptor selective antagonist, cyclopentyl-1, 3-dimethylxanthine (CPT) increased waking and decreased sleep [69,147]. Moreover, single unit recording of basal forebrain wake-active neurons in conjunction with *in vivo* microdialysis of A1 selective agonist, N6- cyclohexyladenosine (CHA) decreased, and A1 selective antagonist, CPT, increased discharge activity of basal forebrain wake-active neurons [148] in a dose-dependent manner [149].

Of particular note, blocking the expression of basal forebrain A1 receptors with microdialysis perfusion of antisense oligonucleotides designed to hybridize with A1 receptor mRNA, and thereby preventing its translation, resulted in a significant reduction in NREM sleep and an increase in wakefulness in the rat (Fig. 9). Moreover, as illustrated in Fig. 10, following microdialysis perfusion of the A1 receptor antisense and 6 h of sleep deprivation, the animals spent a significantly reduced (50–60%) amount of time in NREM sleep during hours 2–5 in the post-deprivation period with an increase in delta activity in each hour [150]. The absence of a sleep stage difference in the first post-deprivation hour suggested that other regions in addition to the basal forebrain, perhaps the cortex, might mediate the immediate sleep response following deprivation. The neocortex is suggested because of the initial deprivation-induced rise in adenosine in the neocortex, but not in other brain regions outside of the basal forebrain. Together, these observations suggested a rather strong site-specific somnogenic effect of adenosine in basal forebrain, with a lesser effect in neocortex. The section on intracellular signaling below describes the A1 selectivity of this pathway.

In contrast to the findings of the A1 receptor knockdown just described, mice with a constitutive A1 receptor knockout did not show a reduced NREM sleep and delta activity following deprivation [151]. Stenberg et al. note that possible determinants of this unexpected finding were the mixed and variable genetic background of the mice and developmental compensation, perhaps with another adenosine receptor compensating. Based on the presence of some overlap in the effects of the A3 and A1 receptor, the authors of this volume suggest the A3 receptor might possibly compensate. An inducible knockout would help obviate developmental compensatory factors.

3.5.2. Receptor mediation of adenosine effects: the A2a subtype and the prostaglandin D2 (PGD2) system

The adenosine A2a receptor subtype mediates sleep-related effects in the subarachnoid space below the rostral basal forebrain, but not in the basal forebrain parenchyma. Here data suggest that there is PGD2 receptor activation-induced release of adenosine which exerts its somnogenic effects by way of the A2 adenosine receptor, as documented in a series of studies by the Osaka Bioscience Institute investigators and collaborators (e.g., [152–156]). Data supporting the somnogenic effects of PGD2 have been reviewed by Hayaishi [156]. PGD2 has been implicated as a physiological regulator of sleep because PGD2 is the major prostanoid in the mammalian brain and the intracere-

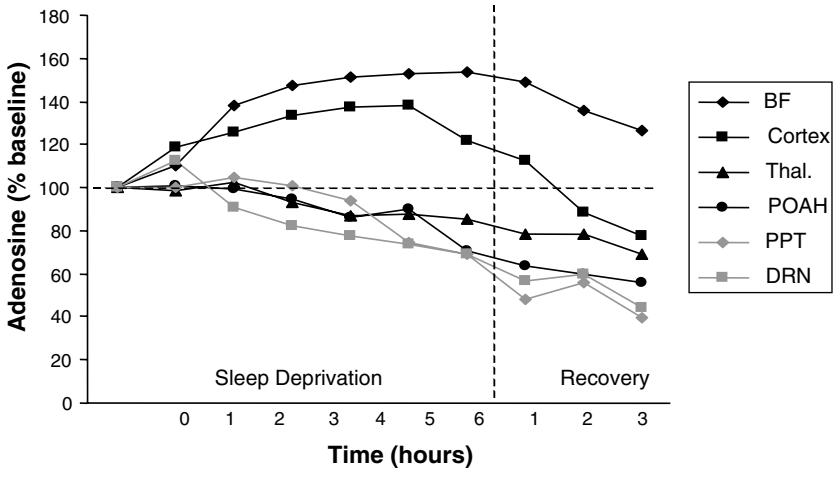

Fig. 9. Adenosine concentrations in six different brain areas during sleep deprivation and recovery sleep. Note that in the basal forebrain (BF, top line), adenosine levels increase progressively during the 6 h of sleep deprivation, then decline slowly in recovery sleep. The visual cortex most closely resembles BF, but adenosine levels decrease during the last hour and fall precipitously during recovery. Other brain areas show no sustained rise in adenosine levels with deprivation. This pattern and other data (see text) suggest that the basal forebrain is likely a key site of action for adenosine as a mediator of the sleepiness following prolonged wakefulness. *Abbreviations.* Thal, Thalamus; POAH, preoptic anterior hypothalamus region; PPT, pedunculopontine tegmental nucleus; DRN, dorsal raphe nucleus. Modified from Fig. 6 in Porkka-Heiskanen et al. [123].

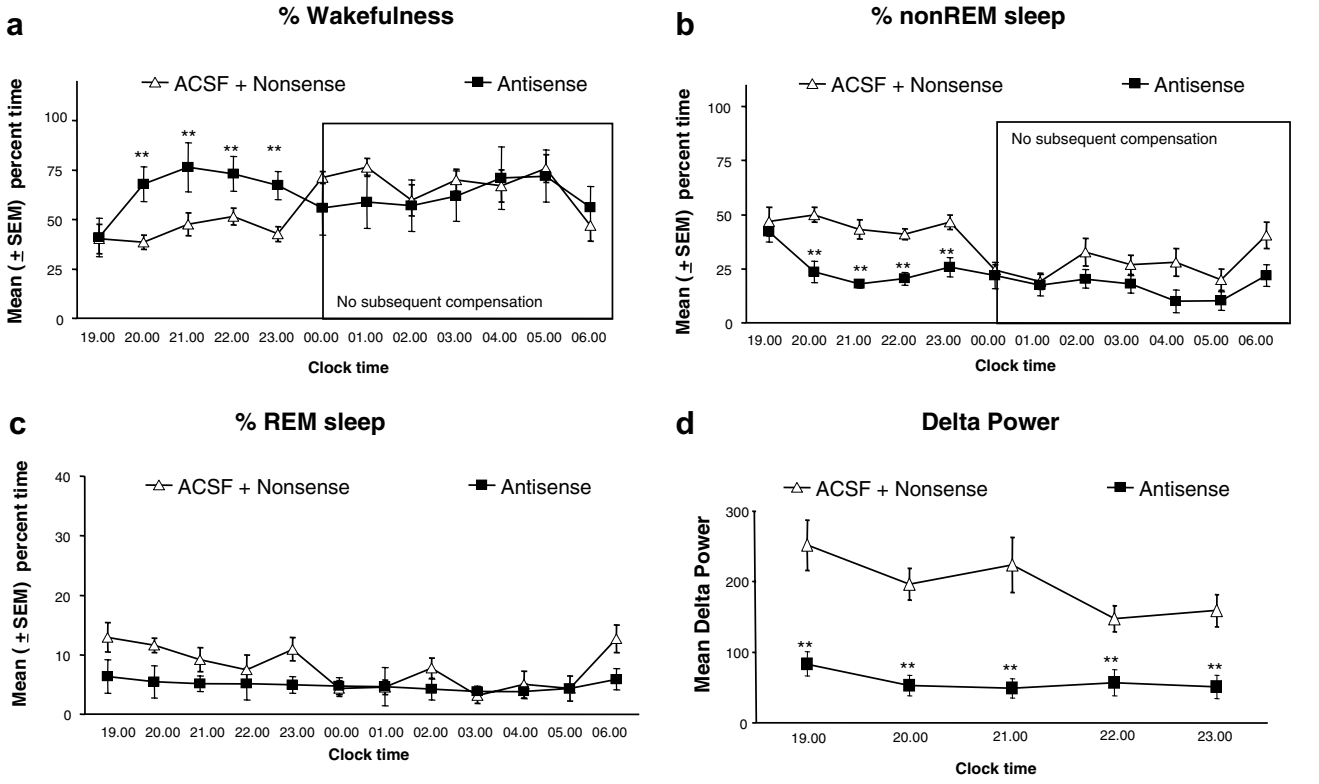

Fig. 10. Effects of basal forebrain perfusion of antisense oligonucleotides against the mRNA of the adenosine A1 receptor compared with controls (ACSF and Nonsense pooled) on recovery sleep following 6 h of sleep deprivation in rats. Note increased wakefulness (a) and decreased NREM sleep (b) during the first 5 h of the recovery sleep period in the antisense group as compared with controls. There was a significant increase in wakefulness and a decrease in NREM sleep during the 2nd, 3rd, 4th, and 5th hour. REM sleep (c) did not show significant differences. The right part of the graphs (within box) shows that, for the subsequent 7 h, there was no compensation for the antisense-induced changes in wakefulness and NREM. Ordinate is mean % time spent in each behavioral state (± SEM) and abscissa is time of day, with lights off occurring at 1900 h and lights on occurring at 0700 h. (d) Differences in delta power (1–4 Hz, mean + SEM) for the antisense and the control group for the first 5 h of recovery sleep. Note the significant decrease in the delta activity in antisense treated animals during each of the 5 h of recovery sleep as compared to the pooled controls ($^{**}p < 0.01$). Adapted from Thakkar et al. [150].

broventlicular (ICV) infusion of femtomolar amounts per minute of PGD2 induced both NREM and REM sleep in rats, mice, and monkeys. Sleep promoted by PGD2 was indistinguishable from natural sleep as judged by several electrophysiological and behavioral criteria, in contrast to sleep induced by hypnotic drugs.

The PGD2 link to adenosine to exert its somnogenic effects is apparently mediated by PGD2 receptors in the leptomenenges in the subarachnoid space ventral to the basal forebrain [154]. Infusion of the A2 agonist CGS 21680 (0.02 to 20 pmol/min) in the subarachnoid space of rats for 6 h during their active period (night) induced SWS in a dose-dependent manner (Satoh et al. [157–159]). Infusion at the rate of 20 pmol/min was effective during the first night but became ineffective 18 hours after the beginning of infusion, resulting in a wakefulness rebound and almost complete insomnia during the first and second days of infusion, a finding attributed to A2 receptor desensitization [160]. These data provide pharmacological evidence for the role of the A2 receptor in mediating the somnogenic effects of PGD2 [157–159]. Moreover, infusion of PGD2 into the subarachnoid space increased the local extracellular adenosine concentration, although dose dependency was not described in this preliminary (abstract) communication [161]. Scammell et al. [155] found robust Fos expression in the basal leptomeninges, as well as the ventrolateral preoptic region (VLPO) of rats treated with subarachnoid CGS21680. The mediator and pathway for leptomeningeal activation of VLPO Fos expression is currently unknown. Scammell et al. [155] speculated, ''Stimulation of leptomeningeal cells by an A2a receptor agonist could induce production of a paracrine mediator that activates nearby VLPO neurons, and studying the effects of PGD2 and A2a receptor agonists on isolated or cultured leptomeningeal cells may help define this local signal.'' These authors suggested that presynaptic inhibition of VLPO might be affected by this paracrine mediator; however, the extant data on presynaptic inhibition of VLPO neurons implicate adenosine [162], and this effect is likely A1-mediated. Scammell et al. [155] noted that data did not support an alternate hypothesis

of A2a effects being mediated by the shell of the nucleus accumbens since, in reviewing the pattern of Fos-IR neurons from previous work with PGD2 [163], they could not identify any change in accumbens Fos expression with infusion of PGD2.

Data indicate that the PGD2–adenosine A2a system plays a special role in pathological conditions affecting the leptomeninges and producing alterations in sleep. Roberts and co-workers [164] reported that the endogenous production of PGD2 increased up to 150-fold in patients with systemic mastocytosis during deep sleep episodes. Subsequently, the PGD2 concentration was shown to be elevated progressively and selectively up to 1000-fold in the cerebrospinal fluid (CSF) of patients with African sleeping sickness [165]. It is possible that the A2aR system is specialized for the mediation of sleepiness that occurs with leptomeningeal inflammation in contrast to the more homeostatically regulated A1 system.

It is useful to mention that in the cholinergic basal forebrain, only A1 but not A2 receptor mRNA (*in situ* hybridization and RT-PCR studies) and protein (receptor autoradiography) have been detected [166]. These data provide strong evidence that in the horizontal diagonal band (HDB) and SI/MCPO regions of the cholinergic basal forebrain the effects of adenosine on sleep–wake behavior are mediated through the A1 adenosine receptor, in contrast to the A2a receptor found in the leptomeninges.

3.6. Adenosine A1 receptor-coupled intracellular signal transduction cascade and transcriptional modulation

Introduction. Prolonged waking or sleep restriction produces progressive, additive effects such as decreased neurobehavioral alertness, decreased verbal learning, and increased mood disturbances, often referred to as 'sleep debt' [167–169]. These effects are cumulative over many days and, thus, unlike the shorter-term effects described in previous sections, are likely to have sleep deprivation- or restriction-induced alterations in transcription as a basis for these long-term effects. Fig. 11 illustrates the adenosine signal transduction pathways that may be responsible for the relevant transcriptional alterations. Basheer, McCarley and colleagues have described an intracellular signal cascade set into motion by the prolonged presence of adenosine, acting at the A1 receptor [4,170]. Briefly, the cascade consists of calcium mobilization from IP3 receptors on the endoplasmic reticulum, activation of the transcription factor NF-κB and its translocation to the nucleus and binding to promotor regions of DNA. Genes whose transcription is controlled by NFkB include the A1R, and there is evidence that this signal cascade results in increased production of mRNA and functional A1R. Interestingly, this signal cascade appears to be confined to cholinergic neurons in the basal forebrain.

Sleep deprivation-induced increase in A1 receptor mRNA and functional A1 receptors in the basal forebrain:

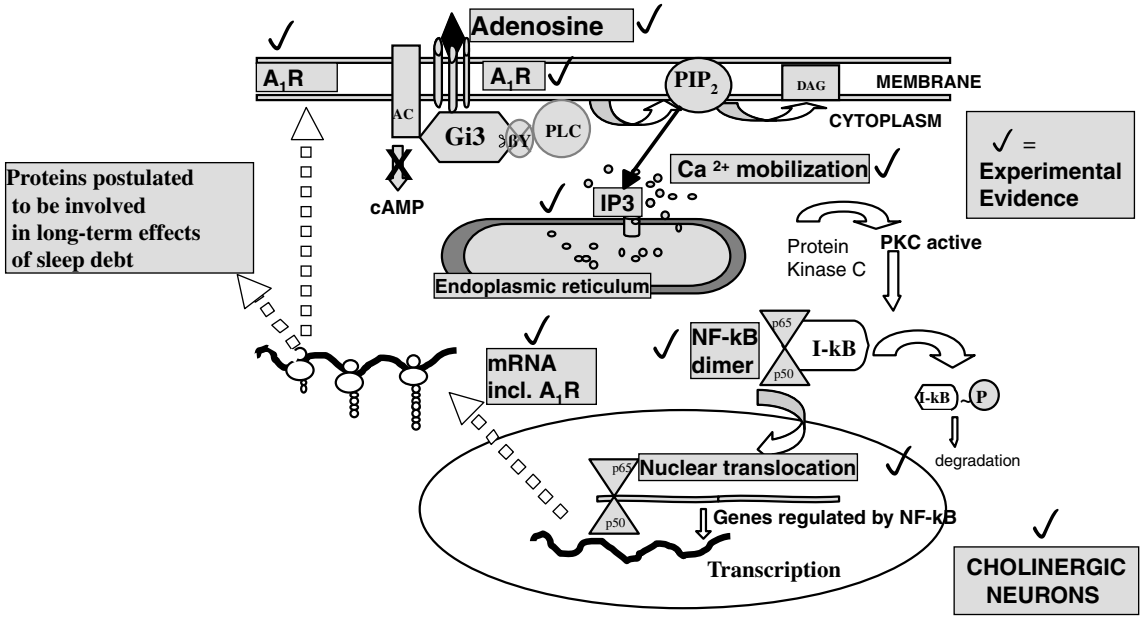

Fig. 11. Model of intracellular signaling pathway of the adenosine A1 receptor in the cholinergic basal forebrain. In brief, adenosine binds to the A1 receptor subtype, proceeds through a second messenger pathway producing IP3 receptor-mediated intracellular calcium increase and leads to an activation of the transcription factor NF-κB. The activated NF-κB translocates to the nucleus and binds to the promotor regions of genes, one of which is the gene for A1 receptor. See text for a description of the steps in the pathway and supporting experimental evidence. The checks in the figure indicate steps for which supporting evidence is present.

resetting the sleep homeostat gain. *In situ* hybridization and reverse-transcription polymerase chain reaction (RT-PCR) of total RNA from the basal forebrain and cingulate cortex showed that 6 h of sleep deprivation resulted in significant increases in A1 receptor mRNA in basal forebrain but not in the cortex [166]. More recent work has shown that longer deprivation, 12–24 h of deprivation, produces a significant increase in functional A1 receptors, as shown by increased ligand binding [170]. It seems clear that prolonged sleep deprivation and up-regulation of the A1 receptor might act to enhance the sleep-inducing effects of a given level of extracellular adenosine concentrations beyond that observed before the deprivation, a ''resetting of homeostat gain'', and positive feedback that would further promote sleepiness.

3.7. Basal forebrain, wakefulness, and adenosine. Cholinergic basal forebrain lesions

As noted above, the basal forebrain has cortically projecting neurons which utilize acetylcholine, GABA, and glutamate as neurotransmitters, in addition to peptides acting as co-transmitters. One of the questions concerns the relative role of each of these neurotransmitters. The intracellular signaling pathway with A1 receptor activation leading to increased A1 receptor production is confined to cholinergic neurons, and, thus, this system is of particular interest. Current findings with respect to lesion effects on the cholinergic neurons differ. As discussed in Kalinchuk et al. [171], the route of administration of the selective cholinergic toxin, 192IgG-saporin, makes a big differences in the results.

When saporin was administered (ICV), there were very small or no effects on sleep ([45,172–175]; Kalinchuk et al., unpublished data). However, when saporin was administered locally into the cholingergic basal forebrain, two separate research groups found, in studies 2–4 weeks post-injection, that spontaneous sleep decreased and recovery sleep and delta activity were both profoundly reduced [176,177]. These similar results from local injections by two independent groups mitigate against technical error causing these findings.

Kaur et al. [177] found 192IgG-saporin injected bilaterally in cholingergic basal forebrain transiently increased NREM sleep time predominantly during the dark (active) phase, with a decrease in recovery delta and recovery SWS time following 6 h of deprivation at four weeks post-lesion. Kalinchuk et al. [176] found local administration (but not ICV administration, unpublished data) of 192IgG-saporin decreased wakefulness and increased sleep. Moreover, recovery sleep and a rise in adenosine levels was abolished after either three or 6 h of sleep deprivation. Adenosine levels in the lesioned animals did not increase during sleep deprivation, nor was there an increase in NO levels. Blanco-Centurion et al. [178] also found that ICV 192IgG-saporin abolished the adenosine rise with sleep deprivation but, unlike the two local administration studies, did not alter recovery sleep. Lu et al. [45] found that basal forebrain cholinergic lesions with either 192IgG-saporin or noncholinergic lesions with low doses of selective lesions of noncholinergic neurons by orexin-saporin did not affect spontaneous wakefulness (deprivation was not studied). A striking finding of total basal forebrain lesions with a higher dose of orexin-saporin was that waking was abolished. Kalinchuk et al. [171] discuss this issue of cholingergic basal forebrain lesions in more detail in a Commentary in the journal *Sleep*.

3.8. Sleep-mediated alterations in behavior: possible relationship to adenosine-induced changes in the basal forebrain cholinergic system

In the basal forebrain, both cholinergic and noncholinergic neuronal activity is associated with promoting wakefulness [87,102,179–182]. The somnogenic effects of adenosine may be due to the inhibition of neuronal activity in both cholinergic and noncholinergic neurons of the basal forebrain. In addition, the modulatory effects of sleep deprivation on the A1 adenosine receptor mRNA and transcription factor NF-κB activation in the cholinergic basal forebrain suggest the significance of an adenosinergic pathway in the long-term effects of sleep deprivation on the quality of ensuing sleep and/or the neurobehavioral alertness, cognitive functions and mood. The cholinergic neurons in the HDB/SI/MCPO target the entorhinal cortex, neocortex and amygdala, and regulate aspects of cognition and attention, sensory information processing and arousal [183–188]. Cognitive functions such as learning and memory show a correlated decline with degenerating cholinergic neurons, as reported in Alzheimer's patients (see reviews in [188–191]). Wiley et al. [192] developed a technique involving 192IgG-saporin-induced lesioning of p75 nerve growth factor (NGF) receptor containing cholinergic cells in rats. The cholinergic lesions using this technique resulted in severe attentional deficit in a serial reaction-time task [193,194]. The cholinergic basal forebrain is important in cortical arousal. Animals with a lesioned basal forebrain show decreased arousal and increased slow waves in the cortex [195,196]. The effects of adenosine on the cholinergic basal forebrain are, thus, potentially important as the related sleep deprivation-induced 'cognitive' effects may be mediated through adenosine.

Obviously, an ability to measure cognitive effects of sleep deprivation in animals would be important. As an initial step, the effects of sleep deprivation on the five choice serial reaction time test (Fig. 12) in the rat have been examined by Cordova et al. [197]. Ten hours of total sleep deprivation produced a pattern of behavioral impairments that were broadly consistent with the

Fig. 12. The 5-choice reaction time test operant chamber. This behavioral chamber contained five evenly spaced ports containing a light stimulus and a sensor that registered nose entry by the interruption of an infrared beam. In each trial, a 0.5-s light stimulus was presented in one of five ports (see illuminated port on the left). A nose poke into the illuminated port within 3 s of the stimulus triggered the delivery of a sucrose pellet into a reward tray in the opposite wall of the chamber that was accessible through a flap door. Sleep deprivation produced an increase in omissions (failure to respond in time), an increased latency, and a decrease in the number of correct responses.

effects of sleep deprivation on vigilant attention performance in humans. Sleep deprivation produced a significant increase in the latency of correct responses in a dose-dependent manner, consistent with a monotonic effect of sleep debt on attention. Sleep deprivation also led to an overall increase in the number of omission errors, during which a rat did not respond to the stimulus within a brief period. The same measures are comparably affected in the psychomotor vigilance task (PVT) following similar deprivation lengths [198]. Thus, the behavioral effects of sleep deprivation closely resemble the findings in human studies using the PVT to assess vigilance and attention deficits after sleep deprivation. In the current task, care was taken to limit possible lapses of performance from sleeping by requiring the rats to behaviorally initiate each trial and by videotape evaluation of behavior.

These effects are also highly compatible with the effects of basal forebrain cholinergic lesions (saporin) in rats [193,194], but direct microdialysis measurements of acetylcholine in rats during sleep deprivation will be needed to prove a relationship with decreased cholinergic activity.

3.9. Adenosine and a model of the consequences of obstructive sleep apnea

In obstructive sleep apnea (OSA) the upper airway collapses during sleep. This has two major consequences: The first is the sleep interruption which prevents individuals from achieving a normal amount of deep (delta) sleep even though there are no conscious arousals; the second is episodes of hypoxemia, whose intensity and number vary between individuals. We had hypothesized that the sleep interruptions might interfere with restorative sleep and that elevations of basal forebrain adenosine might be responsible for some of the cognitive effects. To test the effect of sleep interruption and their relationship to elevated adenosine, we developed a rodent model in which the animals were awakened once every 2 min with 30 s of slow movement on an automated treadmill (see description in [199]). Control rats either lived in the treadmill without movement (cage controls, CC) or had 10-min periods of movement followed by 30 min of nonmovement, allowing deep/continuous sleep (exercise controls, EC). In the sleep-interruption (SI) group, the mean duration of sleep episodes decreased and delta activity during periods of wake increased, compatible with a disturbance of deep sleep. McKenna et al. ([200], unpublished data) found basal forebrain adenosine levels were significantly elevated in the course of sleep interruption compared to both cage and exercise controls. Adenosine rose monotonically during the sleep interruption, peaking at 220% of baseline at 30 h of sleep interruption. The levels with sleep interruption were not statistically different from those during sleep deprivation of the same duration. These data point to adenosine as a causative factor in the sleepiness occurring with obstructive sleep apnea.

Tartar et al. [199] investigated the mechanisms by which sleep fragmentation results in memory impairment. Twenty-four hour SI impaired acquisition of

spatial learning in the hippocampus-dependent water maze test. Moreover, hippocampal long-term potentiation, a long-lasting change in synaptic efficacy thought to underlie declarative memory formation, was absent in rats exposed to 24 and 72 h sleep interruption but, in contrast, was normal in EC rats. Whether increased adenosine in the hippocampus might account for these findings is now under investigation.

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