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ScienceDirect
Metabolism Clinical and Experimental 55 (Suppl 2) (2006) S13–S19
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

The pharmacology of wakefulness

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Abstract

Being awake, alert, and able to function in our 24-7 world is a challenge in the face of the fatigue and sleepiness engendered by long work hours, unusual work schedules, sickness, and other factors. Development of effective treatments to combat fatigue and sleepiness requires an understanding of the neurobiology of wakefulness. In this brief review, we examine the neuroanatomical, neurochemical, and molecular basis of the wakeful state to provide a framework for understanding current and future pharmacologic approaches to modification of wakefulness. The spontaneously awake state can be defined as a natural state of vigilance or arousal differing from natural sleep in both behavior and neural activity. These differences have long intrigued researchers and largely have been characterized in the brain areas and neurochemical systems affecting the sleep and wake states. Many of the strategies for promoting the awake condition involve manipulation or modulation of specific neurochemical systems with the ultimate goal of enhancing wakefulness, diminishing sleepiness, or both. Wakefulness is an important cortical function that depends on the coordinated effort of multiple brain areas including the thalamus, hypothalamus, and basal forebrain to integrate and relay information from the brainstem to the cortex. Norepinephrine and serotonin—long considered arousal-enhancing transmitters as well as glutamate, acetylcholine, histamine, and the neuromodulators hypocretin-orexins and adenosine, are known to affect the signal transduction in these brain areas and initiate, promote, or enhance wakefulness. Use of molecular tools to evaluate the awake, asleep, and sleep-deprived state has revealed novel insights concerning the gene expression events associated with wakefulness. Understanding wakefulness at this level undoubtedly will contribute to the development of pharmacologic approaches to promote or enhance the wakeful state. We caution, however, that sleep may have a necessary, restorative function for the brain; therefore, prolonging wakefulness for long periods through artificial means could have unexpected and perhaps detrimental consequences on brain health.

1. Introduction

As much as we might desire always to be alert and fully functional, the demands of our busy lives and our 24-7 world frequently interfere with this goal. Man has always shown great interest in finding and devising ways to stave off fatigue and sleepiness to stay awake and remain alert. These efforts extend from ancient time, when warriors used various herbal preparations to forestall sleep and allow them to stage prolonged battles, to the modern era where the military uses go/no-go pills during extended flight operations [1]. Thus, there remains an interest in learning how the states of awake and sleep differ, as well for finding ways to initiate or enhance wakefulness. This review examines the pharmacology of wakefulness. To understand how pharmacologic agents or other types of manipulations promote a state of being awake we begin by defining the awake state. To aid in this discussion we briefly outline the brain areas and neurochemicals that initiate or maintain the awake state. The circadian clock located in the suprachiasmatic nuclei provides synchronization to environmental light/dark cycles and plays a role in controlling the sleep/wake cycle, but a discussion of this subject is outside our scope (see Antle and Silver [2] for a review). We also examine how modern molecular techniques have aided in defining the differences between sleep and wake at the cellular level. Finally, as wake-promoting substances and strategies become more effective at maintaining alertness and performance, staying awake for extremely long periods will become common. Nevertheless, we emphasize that there may be unexpected, even negative consequences of prolonging the awake condition because sleep appears necessary for brain health. Thus, we conclude by discussing how artificially prolonged wakefulness may impact the brain.

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0026-0495/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.07.007

2. Defining the awake state

In simple terms one can conclude that being awake is the opposite of being asleep, a fact that emphasizes a crucial point—it is difficult to discuss the awake state without at least referring to sleep. The awake and sleep states often are described in terms of each other; for example, sleep is described as "a successive discontinuation of wakefulness" or "sleep and waking are distinct alternate behavioral states that are mutually exclusive, but interrelated" [3]. Consequently, although sleep is not the focus of this review, it will be discussed when necessary to provide context. Fatigue and sleepiness can occur during the awake state when an individual is deprived of sleep; such deprivation results in deficient performance and cognition, although individual differences exist in how vulnerable one is to sleep deprivation [4]. In general, the need or drive to sleep increases with the length of time one is awake [3,4].

Vigilance and monitoring of the environment are possible during natural waking but not during sleep; a waking state can be maintained whether stimulation is high or low. The loss of these clearly necessary survival behaviors during sleep suggests that sleep is restorative, although its exact function is still debated. Complex cognition also is possible while we are awake. Sleep on the other hand appears vital for "off-line" memory processing or sleep-dependent learning, although the idea that sleep is necessary for memory consolidation is still controversial (see reference [5]). Generally, training followed by sleep but not by a period of wakefulness leads to memory consolidation and better performance on a variety of tasks in humans and experimental animals. This includes tasks involving motor sequence learning, perceptual and visual discrimination, learning of complex logic games, and acquisition of a foreign language, as well as retention of memories associated with emotion [6]. Importantly, both the awake and sleep states require active brain processing; the historical view of sleep, held by Sherrington and others, as an inactive state is no longer accurate. Consciousness wanes as we sleep without a loss in brain activity, although the location and type of activity differ between the 2 states [3,7].

Determining the spectral content of an electroencephalogram (EEG), a record of the electrical activity of the brain first described by the psychiatrist Adoph Berger, is one way in which the waking and sleep states are described and defined in higher organisms [3,8]. Berger noted in the 1920s that low-voltage fast brain waves were apparent in alert, awake humans, but high-voltage, low-activity waves accompanied closed eyes, relaxation, or sleep. All mammals show the same relations between level of arousal and the EEG. Furthermore, the EEG differs between the 2 types of sleep. The non–rapid eye movement (NREM), nondream type is dominated by low-activity waves, whereas the REM or dream type is associated with low-voltage fast waves. Rapid eye movement activity differs from the alert, awake state because sensory input and motor output are inhibited [3]. Because of the advances in positron emission tomography and functional magnetic resonance imaging imaging, these technologies increasingly are used as additional approaches for defining sleep and awake states. Positron emission tomography and functional magnetic resonance imaging do not actually measure brain activity, but measures its surrogates, blood oxygenation or flow, respectively. Although their relationship to brain activity is as yet not fully understood, both imaging techniques suggest, as does the EEG, that broad but reliable changes in cerebral activity occur across the sleep/awake states [7,9]. In practice, the use of polysomnograph data, consisting of the EEG in combination with electromyography and electrooculography (EOG), is considered the most accurate means for determining if a person is asleep or awake. The EEG and EOG are very active during the awake state and REM sleep, but muscle activity is highest when we are awake. During NREM sleep the EEG and EOG are inactive, but there is some electromyography activity [8].

The global states of awake and sleep also have been defined and characterized in a more elemental fashion by examining behavior; that is, if the organism is active, it is assumed to be awake (see Siegel [5] for a discussion of this issue across species). The rest-activity cycle is homeostatically regulated and may be a precursor or primitive form of the sleep/wake cycle. Acceptance of this definition means that even lower organisms that do not possess a well-developed central nervous system are capable of sleep. This also implies the evolutionary significance of sleep and its necessity for primitive life functions [10]. Many investigators now are using lower organisms (eg, the fruit fly) to explore the molecular characteristics of the sleep and wake states, but they still define these states behaviorally because the recording of nervous system activity in these species is difficult at best. For example, wakefulness was defined in fruit flies as any period of 1 or more minutes of activity, because flies immobile for greater than 5 minutes require a stronger stimulus to rouse them [11,12]. Furthermore, wrist activity monitors are used to effectively, but not as accurately, gauge sleep and wake states in humans when a polysomnograph may be impossible or difficult to obtain [8].

3. Brain regions and neurochemicals important for the initiation and maintenance of the awake state

While awake, we are able to monitor our environment, remain vigilant, and act on incoming information. How does this occur and what brain areas initiate and keep us awake? During the awake state, sensory input must be processed, attention directed, memories accessed, posture maintained, and motor movement and forebrain (FB) activity sustained. Clusters of neurons located in the reticular formation (RF) of the brain stem (BS), portions of the midbrain, thalamus (THAL), and hypothalamus (HYPO) with connections to higher brain centers such as the cortex appear to be crucial for these activities (Fig. 1). Groups of cells in these areas are

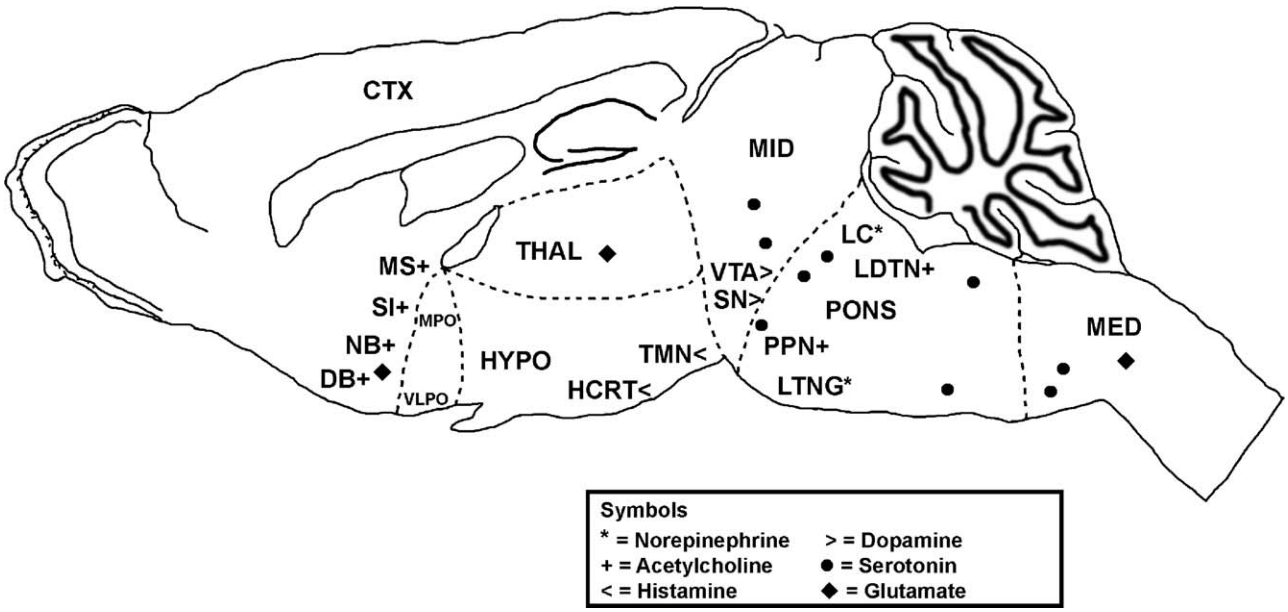

Fig. 1. Brain areas and neurochemicals important in wakefulness.

active and firing while we are awake, but are inactive during sleep [3,10,13].

In the 1940s, Moruzzi and Magoun discovered that the awake state could not be maintained if the RF is damaged; being awake requires FB activation by the BS. The sensorimotor and modulatory neurons in the RF have very specific afferent inputs from receptors in skin, muscle, and other areas of the periphery. Moreover, highly organized outputs of the RF allow integration and transmission of information with resultant appropriate behaviors. Areas of the RF responsible for keeping us awake compete with others devoted to sleep control [3].

The Viennese neurologist Von Economo highlighted the importance of the HYPO (Fig. 1) when he noted that "sleeping sickness" accompanied by pathology of the HYPO often-plagued victims of the encephalitis pandemic in the early 1900s. Lesions in the tuberomammillary nucleus (TMN) of the posterior HYPO were found to promote sleep, whereas insomnia occurred if they were in the preoptic area (POA) of the anterior portion; stimulation of the POA, in contrast, promoted sleep. In addition, other neurons in the anterior HYPO, the ventrolateral POA, project to the posterior HYPO and especially the TMN. The TMN, in turn, projects widely to thalamus and cortex. When active during NREM sleep the ventrolateral POA inhibits the wake-promoting actions of the TMN. These systems of the HYPO are often likened to a sleep/wake "switch" [3].

4. Wake centers and neurochemicals

4.1. Glutamate

The nuclei in the RF serving the wake state are identified by their elaborate connections to higher brain centers. These ascending and descending projections of the RF as well as those of the THAL and basal FB with cortical projections use glutamate (GLU) as a neurotransmitter; other transmitters involved in arousal in turn regulate these GLU neurons. Glutamate is important in cortical activation, arousal, and cognition, and modulating its transmission impacts wakefulness. The ampakine CX717 acts on the AMPA receptor to cause enhanced transmission through the $GLUR_1$ subtype and ameliorates the cognitive deficits caused by 30 to 36 hours of sleep deprivation in subprimates or an overnight loss of sleep in humans. During sleep deprivation, activity of GLU excitatory transmission decreases in brain areas that are normally active, and CX717 restores activity to normal levels [13-15].

Other neurons involved in wakefulness include those that use norepinephrine (NE), serotonin (5-HT), and acetylcholine (ACh) as neurotransmitters. Thus, the awake attentive brain is largely controlled through glutamatergic, aminergic, and cholinergic pathways. Nevertheless, other neurotransmitters and neuromodulators play a role and they include dopamine (DA), histamine (H), and the hypocretins-orexins (HCRTs) [3,13].

4.2. Norepinephrine

The "wake-promoting" neurotransmitter, NE, is vital for an aroused waking state; many aminergic neurons fire spontaneously while we are awake and others increase their output in response to various stimuli. Their activity is greatly diminished or almost nonexistent during NREM and REM sleep. The pons and medulla (Fig. 1) contain major groups of NE neurons, the locus coeruleus (LC) and the lateral tegmental neuron group. The cells of the lateral tegmental neuron group release NE to regulate the HYPO and to control motor behavior, whereas the LC regulate

sensory input and cortical activation. Norepinephrine acts through its receptors to activate areas involved in wakefulness and inhibit those areas devoted to sleep. The role of NE in wakefulness is supported by studies in which adrenergic signaling is altered. For example, genetically altered mice unable to convert DA to NE lack activation at adrenergic receptors, have increased sleep times and are difficult to arouse even when stressed. Because of the large number of NE receptor subtypes, it is difficult to make simple statements about agonist/antagonist action at the NE receptor in relation to wakefulness. Nevertheless, altering the amount of NE at the synaptic site would be predicted to affect wakefulness. Indeed, central nervous system (CNS) stimulants with prominent wake-promotion characteristics block the uptake of NE or cause its release at the synapse. Modafinil is effective in promoting wakefulness in those with narcolepsy and, although it is not a CNS stimulant, recent work suggests it may enhance NE transmission [3,13,16,17].

4.3. Serotonin

The 5-HT–containing neurons of the RF, the medial and dorsal raphe (Fig. 1), contribute to maintaining a quiet awake state; the processes from these clusters of cells innervate the entire FB. These neurons show maximal firing during waking, but no activity during REM sleep. There are many 5-HT receptor subtypes as well as numerous behaviors attributed to 5-HT transmission, factors that likely have contributed to the confusion regarding the role of this neurotransmitter in sleep/wake states. Indeed, evidence exists to support a role of 5-HT both in sleep and wakefulness. However, a consensus has recently emerged that supports a stronger role in wakefulness because promotion of 5-HT transmission (eg, reuptake inhibition, precursor loading, etc) results in quiet nonaroused waking [13,17].

4.4. Acetylcholine

The release of ACh in the RF, HYPO, THAL, and basal FB participates in controlling wakefulness, consciousness, and attention; these areas are innervated by neurons of the laterodorsal tegmental nucleus and pedunculopontine nucleus (PPN) located in the pons (Fig. 1). Other clusters of ACh cells in the basal FB, namely, the medial septum, the diagonal band, the substantia innominata, and the nucleus basalis also are involved in maintaining attention and consciousness through innervation of the limbic areas and cortex. The firing rate of ACh neurons is highest during the wake period, whereas it is minimally active during NREM sleep, observations that support the role of ACh in wakefulness. In addition, activation of ACh receptors by ACh itself, as well as by nicotine, has wake-promoting actions. Consistent with this latter observation, donepezil prevents ACh metabolism, increases ACh levels, and enhances wakefulness. Alzheimer's disease causes basal FB degeneration and is often associated with decreased sleep efficiency and greater nocturnal activity, dysfunctions frequently cited as reasons for institutionalization [3,13,17].

4.5. Histamine

Histamine is considered a wake-promoting substance; a group of H-containing cells in the TMN projects to the FB and BS. These neurons are active when we are awake and are inhibited during sleep by γ-aminobutyric acid (GABA) neurons located in the anterior HYPO (Fig. 1). Lesions in the TMN as well as blockade of H receptors, especially the H_1 receptor, produce somnolence. Accordingly, excessive sleepiness is often an unwanted side effect of antihistamines [3,13].

4.6. Hypocretins-orexins

Neurons containing HCRTs, 2 neuropeptides also known as the orexins, modulate wakefulness and are located in the lateral portion of the posterior HYPO. The release of the HCRTs into the LC and other RF nuclei is required for the sustained activation of these wake-promoting areas and help to stabilize the switch from sleep to wake. The HCRT- and the H-containing neurons of the posterior HYPO together enforce waking. The wake-promoting peptide, HCRT-1, may, in fact, have its actions through the H system as directly injecting it into the TMN causes wakefulness. In humans or animals the loss or dysfunction of HCRT neurons causes narcolepsy, a pathologic inability to remain awake. In normal animals, administering the HCRT peptides enhances monoaminergic transmission and increases wakefulness, possibly through release of GLU. Hypocretins-orexin neurons are silenced during sleep, likely by GABA released from the POA, and this in turn results in less activation of the wake-promoting areas they innervate [3,13,18].

4.7. Dopamine

Dopamine neurons fire at a similar rate during the sleep/wake cycle, suggesting that DA plays a negligible role in wakefulness. The following observations, however, suggest otherwise: (1) DA contributes to waking behaviors involved in movement, motivation, cognition, reward; (2) DA-containing neurons of the substantia nigra (SN) and ventral tegmentum (VTA) as well as DA receptors are important in arousal, and lesioning these areas decreases arousal; (3) neurologic diseases that affect DA neurotransmission, such as Parkinson's disease or restless legs syndrome, are marked by difficulty in maintaining an active awake state, as well as by nocturnal sleep disruption; (4) CNS stimulants, for example, the amphetamines, methylphenidate, pemoline, and cocaine act on DA systems and have arousing effects, they increase vigilance as well as attention and are most effective if they cause DA release; (5) other wake-promoting agents with non-DA mechanisms of action (eg, modafinil, caffeine) may attain a portion of their effectiveness through DA release. These observations suggest that enhancing the actions of DA or making more DA available

in the synaptic cleft will promote wakefulness. Nevertheless, further work is needed to more clearly delineate the role of DA in the wakeful state [1,13,16,17,19].

4.8. Adenosine

It also is possible to increase wakefulness by modulating the systems controlling sleep. Adenosine (A) is a neuromodulator and a sleep-promoting substance; it rises in the extracellular space of basal FB—one of the sleep centers, as wakefulness duration increases and it decreases during sleep. If A mediates sleep, then blocking its effects should enhance wakefulness. Indeed, the caffeine ingested in coffee or in over-the-counter preparations (eg, NoDoz [Novartis]) promotes wakefulness and disrupts sleep by preventing A from being effective at brain receptor sites. Caffeine and other antagonists of A are arousal stimulators, although the exact role of the A receptor subtypes, $A_{2a}R$ and A_1R, even with the aid of data from $A_{2a}R$ KO mice, remains to be defined [16,21]. Caffeine is effective in many low-arousal conditions including sleep deprivation, minor illnesses such as a cold, and the postlunch dip in alertness. Caffeine's effects on wakefulness and on sleep recovery are similar to those provided by intermittent naps. In addition, caffeine is as effective as modafinil when administered on the correct dosing schedule. Caffeine clearly prolongs wakefulness, but does not appear to substitute for the restorative effects of sleep [20-23].

5. Cellular and molecular characterization of wakefulness

Data obtained from neuroanatomical and neurochemical evaluations constitute much of our current understanding of wakefulness. However, new insights into the neurobiology and neuropharmacology of wakefulness are now being obtained based on the results of studies using molecular biologic techniques. For example, the results of genome-wide arrays suggest that there are many genes in the brains of rodents that are more expressed during wakefulness than during sleep, and these genes are different from those that oscillate with the light/dark cycle; that is, being in a wake or sleep state determines the expression pattern for certain genes. Despite the behavioral quiescence of sleep, approximately the same numbers of genes are expressed during the sleep and wake cycles. These data provide additional support for the assertion that both are active states of brain function. Furthermore, these expression changes were specific to the brain and were not found in liver and muscle. Although both states cause increased expression of genes associated with (1) learning/memory and plasticity, (2) cell growth maintenance, and (3) cell signaling, being awake favors different cellular processes. Wakefulness favors synaptic plasticity events associated with acquisition and synaptic potentiation, rather than consolidation and synaptic depression. Wakefulness also favors gene expression patterns related to metabolic processes commensurate with high-energy demands, for example, glucose metabolism, as well as a greater cellular stress response, as evidenced by an increased expression of genes coding for chaperone and heat shock proteins. Finally, wakefulness shows a strong association with events related to excitatory synaptic transmission such as GLU synthesis, membrane depolarization, and synaptic activity as well as the positive regulators of transcription rather than those related to GABA-mediated inhibitory transmission. Wakefulness shows minimal impact on gene expression events associated with glial function, membrane synthesis, maintenance and trafficking or synthesis/transport of cholesterol, or the translation of messenger RNA. Array studies examining the sleep and wake states in fruit flies have found similar patterns, results suggestive of an evolutionary conservation of cellular processes specific to the sleep/wake state [11,19,24,25].

6. Prolonging wakefulness through artificial means—are there negative consequences for brain health?

Continuous or intermittent bouts of prolonged wakefulness simultaneously involve a decrease in the number of hours spent in sleep and a consequent deprivation in sleep. The intensity of sleep after deprivation is directly related to the duration of wakefulness, suggesting that sleep is a necessary function and that the response to this deficit is compensatory. However, restful wake periods do not substitute for sleep; although naps can ameliorate some of these deficits, society tends to discourage the use of naps to contend with the effects of sleep deprivation. Consequently, pharmacologic agents with wake-promoting properties have been sought to combat the common debilitating effects of sleep deprivation, including feelings of fatigue, a deterioration of mood, as well as deficits in cognition and decision-making. The currently available agents, caffeine, modafinil, and CNS stimulants, can be effective in some but not all sleep-depriving situations (eg, situations as diverse as military sorties to shift work). These agents have limitations to varying degrees ranging from cost to problematic side effects; therefore, the pressure to develop better and more effective wake-promoting agents is likely to continue [16,22,23]. As genomic and proteomic advances continue to aid in the characterization of the sleep/wake states, these technologies will contribute to the identification of therapeutic targets susceptible to manipulation by pharmacologic interventions.

The ideal wake-promoting agent would provide a period of wakefulness when desired. Performance would be maintained despite a lack of sleep, subjective symptoms such as sleepiness would be diminished, and the quality of subsequent sleep would not suffer due to drug administration. Being able to eliminate the discomforting and debilitating aspects of limited sleep will likely lead to increasing use of wake-promoting agents as "lifestyle" aids [14,26]. But will brain health suffer as newer and more

effective wake-promoting agents are developed that allow us to remain awake continuously for days and even weeks? Several lines of evidence suggest that prolonged wakefulness has the potential to be detrimental.

The function of sleep is still debated but, as discussed above, it is an active state involving cellular processes and functions distinct from but often complementary to those of wakefulness. Recent work suggests that the processes occurring during sleep may be instrumental to health and survival. The fruit fly "minisleep" mutant shows limited rest with apparently no immediate physiological or behavioral impairment; however, this and other "short-sleep" phenotypes are always accompanied by a truncated life span [27]. Whether shortened sleep affects life span in humans is unknown. Shift work often results in sleep deprivation and is associated with both general health disturbances as well as higher mortality from stroke, suggesting an effect on overall brain health [28].

The cellular processes and functions of spontaneous or natural wakefulness involve excitatory transmission and other functions with high cellular demands that can lead to cellular stress. In support of this, natural wakefulness in the rodent and fruit fly causes the induction of genes coding for chaperone and heat shock proteins; these proteins aid in counteracting cellular stress and protect the organism during challenging environmental conditions. The artificial wakefulness of sleep deprivation also causes induction of these genes, and mutant flies incapable of inducing these stress response genes in response to sleep deprivation will die [12]. In the mouse, 6 or more hours of sleep deprivation induces regulatory proteins essential in the unfolded protein response, a regulatory pathway involved in counteracting cellular stress. The correct folding of proteins is both highly controlled and essential for brain health; abnormally folded or aggregated proteins are a characteristic of many neurodegenerative diseases [29]. It is not known if the wakefulness produced by pharmacologic interventions or certain other artificial conditions will induce cellular stress responses at levels sufficient to overwhelm these endogenous coping mechanisms.

In dogs, widespread neurodegeneration has been reported after the total sleep deprivation induced by forced walking, with attendant stress and exhaustion [30]. In contrast, recent work using less stressful sleep deprivation procedures in rodents has found little or no evidence of widespread neuronal damage or loss [31,32]. Sleep deprivation has also been found to be protective against the neural damage caused by stroke; however, neurons in sleep/wake processing areas may be reduced in size or show signs of degeneration after deprivation [30,33]. Moreover, even limited sleep deprivation reduces learning-induced hippocampal neurogenesis [34]. In the future, it will be critical to determine whether prolonged wakefulness induced by pharmacologic agents results in indirect effects as severe as neurodegeneration to as subtle as an inhibition of neurogenesis.

7. Summary

Anatomical and biochemical studies have revealed brain wake centers and their neurochemical makeup. These include (1) the GLU neurons in the RS of the BS, THAL, and basal FB; (2) the NE neurons of the LC; (3) the DA neurons of the SN and ventral tegmental area; (4) the 5-HT neurons of the BS raphe; (5) the ACh neurons of the pons and basal FB; and (6) the H and HCRT neurons of the HYPO. The overlap and redundant functions (eg, cortical activation) of these systems suggest that no one brain area or neurochemical is solely responsible for the awake state, although each makes a unique contribution to wakefulness. Although forced wakefulness or extreme sleep deprivation is known to be debilitating, there is little detailed information on the cellular and molecular consequences of prolonged wakefulness when it is induced by different means. However, molecular studies have revealed that processes associated with energy demand, synaptic excitatory transmission, transcriptional activity, and cellular stress are high in the brain during normal wakefulness. The advances in genomics and proteomics will aid in determining how these processes are impacted when prolonged wakefulness is achieved in various ways. These techniques will also be useful in identifying new targets for the development of wake-promoting agents.

Acknowledgment

The authors thank Dr Stanley A. Benkovic for his expert assistance with the graphics.

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