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Sleep, a functional enigma

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Abstract

Although continued total sleep deprivation is fatal, the function of sleep remains a mystery. Shorter durations of sleep deprivation are followed by rebound increases in non-rapid eye movement (nonREM) sleep, suggesting a homeostatic control. Measurements of the power spectrum of the EEG suggest that a more accurate marker of the homeostasis may be delta frequency power, as it most closely reflects the duration of the preceding sleep deprivation. Several lines of evidence suggest a link with complex metabolic processes. These include a local homeostatic factor, adenosine**,** that inhibits neuronal activity in response to increases in the ratio of energy demand to metabolite availability. Other evidence derives from the relationship of circadian genes, NPAS2 and Clock to metabolism. Additionally, at a systems level, hypocretin/orexin may coordinate motor activity with feeding. A loss of hypocretin neurons or a mutation of the genes controlling this peptide system can result in the sleep disorder, Narcolepsy.

Finally, evidence for a role of non-REM sleep in developmental CNS plasticity as well as learning and memory is discussed.

The function and control of sleep in animals remains one of the most elusive enigmas of biology. Because the behavioral manifestations of sleep that characterize this state, including motor inactivity, decreased or delayed responsiveness to sensory stimuli and rapid return to normal waking levels of these behavioral parameters with arousal(Shaw et al., 2000;Shaw and Franken, 2003), are dependent on altered CNS activity in vertebrates and probably, invertebrates as well, sleep is, in particular, an enigma of neurobiology. In higher vertebrates this characteristic change of behavior is correlated with a change in the pattern of activity of the majority of neurons in the CNS as suggested by an altered EEG waveform. The waking EEG shows predominantly low amplitude but high frequency components compared to a sleeping EEG (the non-Rapid Eye Movement or non-REM portion) with predominantly high amplitude components especially in the 0.5-5Hz frequency range. The other neuronal characteristic of non-REM sleep is a slowing of the CNS action potential firing rates compared to waking levels (Evarts, 1967). In addition, mammals also have CNS state transitions from non-REM to REM sleep. REM sleep is similar to waking with respect to the firing rates of most neuronal cell types**,** as reflected by the EEG. REM is dramatically different in that this "waking-like" EEG activity is associated with a profound somatomotor inhibition and a reduced responsiveness to sensory stimuli. Neither the reason for these changes (i.e. the function subserved by them) nor the neurobiological mechanism(s) responsible are understood, but some potentially important clues constraining these questions of why and how have emerged from recent studies as described below.

Sleep deprivation

The traditional approach employed in the investigation of the function of sleep has been to examine the outcome of sleep deprivation, but even this seemingly straightforward approach is not without controversy. Much of it stems from the phenomenological definition of non-REM sleep as a group of temporally associated phenomena that usually includes an EEG with predominant slow wave activity and reduced sensory and motor activity as indicated by a less active EMG and reduced responsiveness to sensory stimuli. While this group of phenomena is fairly distinct between normal or average behavioral states of waking and non-REM sleep, when perturbations from the norm occur, then the distinctions break down. This happens with severe sleep deprivation, brain lesions or pharmacological manipulations, as for example, the intrusion of delta wave activity into the waking EEG. Further, these distinctions cannot be directly applied to non-mammalian species since the EEG and EMG are qualitatively different for waking and non-waking behavioral states.

One important indicator for sleep function is suggested by the rebound increase in sleep that follows sleep deprivation. Although the relationship between sleep and the preceding sleep deprivation is variable, the relationship of the slow wave activity during sleep following sleep deprivation (as measured using a Fast Fourrier analysis of the EEG power in the delta frequency range of 0.5-4Hz) is correlated with the duration of sleep deprivation (Franken et al., 1991;Borbely, 1982;Tobler and Borbely, 1986). This relationship is even more robust if one focuses entirely on the delta power deprivation and rebound (Franken, et al, 1991). The delta power increase results from a combination of increased activity and synchronization of CNS electrical activity within the delta frequency range. At a cellular level, this probably reflects a coordinated burst-pause

firing in the thalamus and cortices. The burst-pause oscillation is intrinsic to many thalamocortical neurons, and is mediated by an interaction of the hyperpolarization-activated current (Ih), the transient calcium current (It) and a resting membrane potential in the absence of Ih of ~-80mV(McCormick and Pape, 1990;McCormick and Bal, 1997). The question still remains whether this is only an indicator of sleep homeostasis or rather a mechanism that serves an integral role. Predictably, the burst-pause activity will be associated with a characteristic oscillation, at the delta frequency, of intracellular calcium concentration. It is conceivable that this particular intracellular calcium dynamic signals or at least modulates gene expression and/or second messenger activity.

What happens with prolonged total sleep deprivation? Rats develop a progressively more debilitated appearance, skin lesions and increased energy expenditure until 11-32 days of deprivation at which point they die (Everson et al., 1989). Although the energy expenditure is increased, the food intake is also increased and overall energy utilization was not significantly changed nor are glucocorticoid and thyroid hormonal levels (Bergmann et al., 1989). Gene array analysis shows an altered expression of a number of genes, mainly involved in intermediate carbohydrate metabolism, protein metabolism and mitochondrial organogenesis and function (Cirelli and Tononi, 2000;Cirelli, 2002;Terao et al., 2003). Surprisingly, with prolonged sleep deprivation, most of the upregulated gene expression return to baseline or are slightly depressed with the exception of Arylsulfotransferase in the mammal or its homologue in Drosophila (Cirelli and Tononi, 2000;Tranque et al., 1996;Cirelli, 2002). Further, there is no evidence from the TUNEL technique of cellular degeneration following up to 14 days of sleep deprivation (Cirelli et

al., 1999), although increased amino cupric silver staining occurs, indicative of **a** loss of membrane integrity(Eiland et al., 2002)**.**

A major advance in our understanding of sleep function utilized a Drosophila melanogaster model of sleep (Shaw et al., 2000;Hendricks et al., 2000). Quiet rest (QR) cannot be distinguished from non-REM sleep with traditional means, of course since the EEG measure of increased slow wave (in the delta frequency range) activity cannot be measured. However, whatever CNS arousal is necessary to sustain the coordinated somatomotor and sensory activity needed to respond to the "gentle tapping or shaking" used to prevent rest is also sufficient to elicit a homeostatic rest rebound, similar to the sleep or delta activity rebound observed in mammals in response to sleep deprivation.

Sleep and circadian factors

Shaw and his colleagues have shown that Drosophila with a single mutation of the circadian gene, cycle (cyc; a homologue of the BMAL circadian gene in mammals) or of the heat shock protein (Hsp83) had a strikingly altered response to QR deprivation(Shaw et al., 2002). The rebound response was greatly exaggerated and, if the deprivation was prolonged, then the effects were lethal as compared to no lethality in wild type flies. Finally, pre-exposure to heat was protective against the lethal effects of sleep deprivation in cyc mutants. Thus, a rest/activity cycle homeostasis was demonstrated for non-vertebrates, controlled by specific genes with vital function, at least with respect to the survival of sleep deprivation. Perhaps, of even greater import was that a functional interaction between circadian and homeostatic controls of rest/activity is suggested by these findings.

The interaction between circadian and homeostatic mechanisms of rest/activity control may also be affected by the NPAS2 transcription factor. In the forebrain of mammals, NPAS2 acts as a functional analogue of the circadian gene, Clock, and, either Clock or NPAS2 may bind to form a heterodimer with BMAL1 to activate transcription of downstream circadian gene products, including Per1, Per2 and Cry1 (Reick et al., 2001). However, the activity of either Clock-BMAL1 or NPAS2-BMAL1 heterodimer is under tight control of the redox state of the cell (Rutter et al., 2001;Rutter et al., 2001). Its activity is greatly facilitated by the reduced state of NADP(H) and NAD(H) and inhibited by the oxidized state. Since the redox state is affected by neuronal activity, prolonged neuronal activity might be expected to feedback onto circadian controlled activities. In fact, sleep deprivation by gentle handling in the absence of increased exercise, was shown to reset circadian phase in the Syrian Hamster, possibly in relationship to the prolonged increased neuronal activity of waking and an associated altered redox state (Antle and Mistlberger, 2000). Thus, prolonged sleep deprivation might contribute to a disruption of the normal circadian progression of gene expression under the control of the NPAS2/BMAL1 dimer that, in the absence of prolonged waking, occurs in accordance with the expected circadian rest/activity cycles.

Adenosine

Adenosine is another potential factor that might mechanistically link metabolism to neuronal sleep activity. The extracellular adenosine concentration increases whenever the ratio of metabolite demand to metabolite availability is increased. The increased concentration can inhibit neuronal activity through adenosine A1 receptors, reducing

metabolic demand, and thus providing a negative feedback homeostasis between metabolic demand and neuronal activity (Greene and Haas, 1991). In addition, A1 receptor activity may facilitate delta frequency oscillations (figure 1) by: 1) a combination of increased GIRK **(**G-protein dependent inwardly rectifying potassium**)** channel conductance and decreased activation of the hyperpolarization-activated current, Ih (Pape, 1992); 2) presynaptic inhibition resulting in a functional deafferentation of the thalamocortical circuits that increases the influence of the GIRK and Ih currents to facilitate delta frequency oscillations and; 3) a reduction of cholinergic tone (which inhibits burst-pause firing patterns(McCormick, 1993) by acting at both the cholinergic nuclei (Rainnie et al., 1994;Portas et al., 1997;Porkka-Heiskanen et al., 1997) or locally at the terminal fields of the cholinergic thalamocortical targets (Materi et al., 2000). As noted above, this delta frequency activity correlates strongly with sleep homeostatic response to sleep deprivation, and it is conceivable that this kind of neuronal activity is especially facilitated in regions of high adenosine concentration resulting from increased neuronal activity. With sleep deprivation and the associated increase in duration of the neuronal of waking, the extracellular concentration of adenosine increases in the thalamus (Porkka-Heiskanen et al., 1997) and in the cortex (Porkka-Heiskanen et al., 2000). The central nervous system state of non-REM sleep might be considered permissive to delta wave activity that would be facilitated by the increased adenosine. Others have speculated that adenosine's role in sleep relates to both delta wave activity (Benington et al., 1995) and its induction of glycogenolysis since glycogen stores may be depleted during prolonged waking (Benington and Heller, 1995;Kong et al., 2002). This

is consistent with adenosine's role in energy metabolism to induce glycogenolysis under conditions of increased neuronal activity (Magistretti et al., 1986).

Hypocretin (also called orexin)

Another link between sleep and metabolism is suggested by the fasting induced arousal observed in mammals. Yamanaka et al.(Yamanaka et al., 2003) have reported that hypothalamic Hcrt neurons regulate arousal according to energy balance in studies of Hcrt knockout mice. They find that these mice fail to respond to fasting with increased activity and wakefulness. In prior studies Wu et al. (Wu et al., 2002) reported that Hcrt levels were not altered by 48 hour food deprivation nor by food consumption in normal dogs. However, when these same dogs were allowed to exercise in a large yard, Hcrt levels increased by an average of 70%. These observations can be reconciled by the hypothesis that Hcrt is more closely linked to aspects of motor activity (and the associated neuronal activity) than to peripheral energy balance *per se* and that food deprivation in mice under the conditions of Yamanaka et al.'s studies increased motor activity whereas it did not under the conditions of Wu et al.'s study.

Many of the symptoms of the human disease, narcolepsy can be mimicked by genetic deletion of the hypocretin or orexin peptide in mice (Chemelli et al., 1999) or the mutation of the hypocretin or orexin receptor2 gene in the dog (Lin et al., 1999). In 2001 it was discovered that most human narcolepsy was caused by a loss of hypothalamic cells containing the peptide hypocretin (Hcrt, also called orexin) (Peyron et al., 2000;Thannickal et al., 2000). It was also found that administration of the peptide to genetically narcoleptic dogs reversed symptoms of the disorder (John et al., 2000),

suggesting that similar treatment of human narcoleptics could be a uniquely effective treatment for narcolepsy.

In further work in normal animals, it was found that Hcrt was released maximally in waking and REM sleep and minimally in nonREM sleep (Kiyashchenko et al., 2002). In waking, its release was linked to certain kinds of motor activity leading to the hypothesis that release of Hcrt facilitates motor activity during emotionally charged activities of the sort that usually trigger cataplexy (Wu et al., 2002;Gulyani et al., 2002;Eiland et al., 2002;Siegel, 2003a). Even normal individuals experience weakness at these times, seen in the "doubling over" that often accompanies laughter or the weakness that can result from sudden onset, strong emotions. In the absence of the Hcrt mediated motor facilitation, muscle tone is lost at these times. Many Hcrt neurons also have ascending projections to link cortical arousal to motor and emotional activity. In the absence of Hcrt mediated facilitation of forebrain arousal centers, waking periods are truncated, resulting in the sleepiness of narcolepsy (Siegel, 2003b;Siegel, 1999;Siegel, 2003a)

Hcrt appears to act largely by modulating the release of amino acid neurotransmitters (van den Pol et al., 1998). Systemic injection of Hcrt causes a release of glutamate in certain regions innervated by Hcrt, producing a potent postsynaptic excitation (John et al., 2003;Peever et al., 2003). In other regions it facilitates GABA release producing postsynaptic inhibition (Liu et al., 2002;Kiyashchenko et al., 2002). The loss of these competing inhibitory and facilitatory influences appears to leave brain motor regulatory and arousal systems less stable than the tightly regulated balance that can be maintained in the presence of Hcrt (figure 2). According to this hypothesis, this loss of stability is

the underlying cause of narcolepsy, with the result being inappropriate loss of muscle tone in waking, inappropriate increases of muscle tone during sleep resulting in a striking increased incidence of REM sleep behavior disorder in narcoleptics (Schenck and Mahowald, 1992). In the same manner, although a principal symptom of narcolepsy is intrusions of sleep into the waking period, narcoleptics sleep poorly at night with frequent awakenings (Siegel, 1999;Guilleminault and Anognos, 2000;Siegel, 2000). In other words, narcoleptics are not simply weaker and sleepier than normals. Rather, their muscle tone and sleep-waking state regulation is less stable than that in normals. We hypothesize that this is due to the loss of the simultaneous inhibitory and excitatory actions of Hcrt.

Sleep, plasticity, and learning and memory

Sleep deprivation has been shown to alter cortical plasticity during development. Occlusion of one eye in the cat for as little as 6 hours results in significant plastic changes in the visual thalamocortical circuitry during a critical developmental period for ocular dominance. Sleep deprivation reduces the remodeling induced by the monocular occlusion (Frank et al., 2001). As the authors note, this finding does not imply a particular mechanism for the sleep deprivation effects, nor was it possible to correlate the amount of sleep with the degree of plasticity (altered response). More importantly, it cannot be determined whether the sleep induced reduction reflects a direct effect on mechanisms responsible for the remodeling, or some indirect effect permissive to the remodeling process. For example, this process is known to depend upon protein synthesis(Taha and Stryker, 2002), so that any changes in protein metabolism might have

an impact. Similar concerns may apply to the interpretation of the effects of sleep deprivation on consolidation or reconsolidation of a procedural learning and memory task.

In humans, memory can be subdivided into declarative and procedural forms. Declarative memory is fact based information of the sort that we learn in school or information acquired during daily activities. Declarative memory has been found to utilize the hippocampus and adjacent temporal lobe structures and these structures have therefore been the focus of physiologically based sleep-learning experimenters, including those observing neuronal unit activity during sleep (Louie and Wilson, 2001). Procedural memory is a non-conscious learning such as improvement of perceptual or motor skills as might occur in learning to ride a bicycle or to play a musical instrument

Initial sleep learning studies revolved around that concept that dream activity represented a rehearsal of declarative tasks and that REM sleep was critical to declarative memory. However, accumulated evidence demonstrating that neither REM sleep nor total sleep loss interfered with learning when non-stressful sleep deprivation was employed, has led critics to question the evidence for a role for sleep in declarative learning (Siegel, 2001;Vertes and Eastman, 2000). Recently, even a leading advocate of a role of sleep in learning has concluded that there appears to be no substantial role for either REM or nonREM sleep in declarative learning (Smith, 2001).

Therefore, most current sleep-learning work has focused on the idea that sleep may have a role in procedural learning. However, evidence for an important role of sleep in procedural learning has also been inconsistent. Studies supporting a role for sleep in the consolidation of human procedural learning have made contradictory claims about similar learning tasks, with some concluding that REM but not nonREM sleep is important,

others stating just the reverse, yet others claiming that both sleep states are essential (Stickgold et al., 2000b); Siegel, 2001) and still others making ad hoc claims, such as that only stage 2 nonREM sleep in the last quarter of the night is important (Walker et al., 2002), disputed by procedurally similar studies claiming that REM sleep rather than stage 2 was important (Fischer et al., 2002). Therefore, despite great interest in the area, the evidence for a sleep-learning connection remains weak. Nonetheless, it remains a curious observation that if a subject is allowed to sleep in the hours following training (8 hrs for example) on certain simple sensory motor tasks the performance improves despite the absence of any further training (Walker et al., 2003;Stickgold et al., 2000a). Further, performance on this task following a re-exposure to it, can also be affected by subsequent sleep or the lack of it. The mechanisms that might be responsible remain to be examined. This may be a form of consolidation and/or re-consolidation but the mechanisms are clearly quite distinct from the consolidation or re-consolidation of either, episodic (or in humans, declarative) or emotional, learning and memory since different neuroanatomical systems are involved with procedural learning and memory. A phenomena that could involve procedural memory systems is the increased cortical responsiveness observed in non-REM sleep following slow (~one hertz or less) oscillations that lasts for minutes (Steriade and Timofeev, 2003), although the involvement of plasticity lasting for several minutes should not be confused with consolidation, itself. Furthermore, the existence and characterization of consolidation and reconsolidation processes for these kinds of procedural memory is not well established.

Neurons located in the hippocampus fire action potentials in correlation with an animal's location (hence are termed place cells). Remarkably, the correlation of the firing of

multiple place cells changes with repeated exposure to and exploration of a particular spatial environment like a maze, and these changed correlations of firing may be observed during sleep (Wilson and McNaughton, 1994;Skaggs and McNaughton, 1996) in conjunction with sharp wave activity in the hippocampus (Lee and Wilson, 2002;Ylinen et al., 1995;Nadasdy et al., 1999). It is noted that the relationship between place cells and spatial learning is not well understood (McHugh et al., 1996) much less the relationship of this kind of correlated firing of multiple place cells. Nevertheless, these kinds of activity are consistent with a permissive or facilitative role of sleep in spatial memory but the sharp wave activity is not restricted to sleep(Buzsaki et al., 1992). This hippocampal activity does raise the possibility of a role in consolidation of episodic memory traces(Csicsvari et al., 2002;Nadasdy et al., 1999;Buzsaki, 1998). What is clear, is that adequate sleep is vital to the performance of a wide range of tasks, whether these tasks are well consolidated or recently learned.

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Figure Legends

No figure 1 legend

Figure 2 legend

Lines terminated by perpendicular lines denote excitation; circular terminations indicate inhibition. ACH, acetylcholine; DA, dopamine; NE, norepinephrine; 5HT, serotonin; OB, olfactory bulb; Acb, nucleus accumbens; f, fornix; OX, optic chiasm; CM, centromedian nucleus of the thalamus; PH, posterior hypothalamus; VM, ventral midbrain; AP, anterior pituitary; SC, superior colliculus; IC, inferior colliculus; DR, dorsal raphe; LDT, laterodorsal tegmental and pedunculopontine; LC, locus coeruleus; CBL, cerebellum.

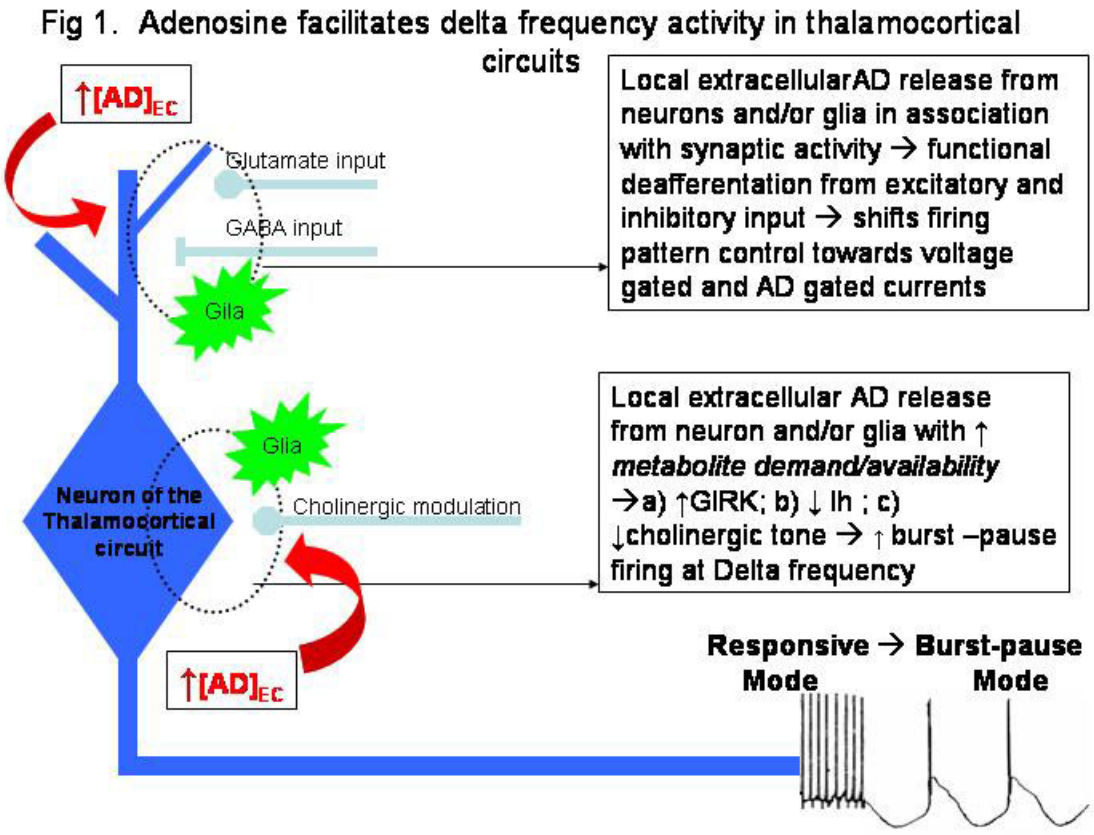

Figure 2 Major identified synaptic interactions of Hcrt neurons.