Humoral Regulation of Sleep; Implications for Sleep as an Emergent Use-Dependent Local Process

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ABSTRACT

Sleep is regulated in part by substances that are produced in response to wakefulness cellular activity. These substances in turn induce state changes in neural networks. Several of these sleep regulatory substances are well-characterized and include tumor necrosis factor (TNF), interleukin-1, adenosine, prostaglandin D_2, and growth hormone releasing hormone. This review is focused on TNF -sleep mechanisms. ATP co-released during neurotransmission induces the release of TNF from glia via purine P2 receptors. TNF acts directly on neurons to alter membrane potentials and gene expression of adenosine and glutamate receptors. Changes in the number of these receptors on neurons alter the sensitivities of the neurons. Thus the input-output relationships of the networks within which the neurons are located are changed. We posit that these events lead to state oscillations and these changes are thus use-dependent and local. The concept that cortical assemblies such as cortical columns oscillate between states has broad implications for sleep regulation, pathologies and function.

Sleep deprivation is associated with enhanced sleepiness, sleep, and fatigue, increased sensitivity to pain and to kindling stimuli, reduced memory and cognitive function, and performance impairments. Chronic sleep loss is also associated with pathologies such as metabolic syndrome, chronic inflammation and cardiovascular disease. Multiple disease states are characterized by increases in sleep, sleepiness and fatigue including; sleep apnea, insomnia, excessive daytime sleepiness, AIDS, myocardial infarction, pre-eclampsia, post-dialysis fatigue, alcoholism, chronic fatigue syndrome, post-viral fatigue syndrome, rheumatoid arthritis and influenza viral infection. These disease states and sleep loss are all associated with enhanced circulating levels of cytokines such as tumor necrosis factor alpha (TNF) and interleukin-1 (IL1) [1]. There is also animal literature implicating these cytokines in physiological sleep regulation and in the sleep responses occurring after

sleep loss and in disease states [2]. Further, injection of IL1 or TNF induces all of the symptoms associated with sleep loss. The current review emphasizes some of the animal data implicating brain TNF in sleep regulation. Those data will then be used to develop the idea that sleep is a fundamental process of neuronal assemblies (e.g. cortical columns) that is use-dependent. The review will conclude with a brief statement as to what this means for sleep function.

Humoral regulation of sleep; emphasis on TNF

We have known for about 100 years that the transfer of cerebrospinal fluid from an animal deprived of sleep to a normal rested animal enhances sleep in the latter [2]. This finding has been replicated many times and has led to the discovery of several sleep regulatory substances (SRSS) such as TNF, IL1 and others (see Table 1). Many investigators [3-6] have proposed criteria that the SRS should meet, e.g. it should enhance sleep, if inhibited reduce spontaneous sleep, vary in brain with sleep propensity, act on sleep regulatory networks and vary with pathology-associated changes in sleep/sleepiness. These criteria are necessary because it is not possible to isolate sleep as an independent variable. New approaches such as genome-wide searches for transcripts that change with sleep or sleep loss have been successful in identifying new candidate SRSS although these new candidates have yet to be validated via the criteria mentioned. The changes observed for some of these substances are likely a consequence of sleep/sleep loss rather than a reflection of direct involvement in sleep regulation. Regardless, it is now recognized that the humoral regulation of sleep is complex, involving many substances (Table 1) that interact in pathways involving multiple cell types within the brain over time courses ranging from days and hours to milliseconds (Figure 1). What follows is focused only on TNF; it is recognized that similar data exist for the other substances in Table 1 [2].

Central or systemic injection of TNF enhances duration of NREMS. For example, intraperitoneal injection of TNF into mice results in about 90 minutes of extra non-rapid eye movement sleep (NREMS) during the first 12 hours post-injection without greatly affecting duration of REMS. In contrast, inhibition of TNF using either antibodies or the soluble TNF receptor reduces spontaneous NREMS and attenuates the NREMS rebound occurring after sleep deprivation and the enhanced NREMS accompanying acute mild increases in ambient temperatures [7]. Mutant mice lacking the TNF 55 kD receptor or those lacking both the 55 kD and the 75 kD receptors sleep less than control mice [8].

Table 1. State regulatory substances meeting all the criteria for such substances outlined in references 3-6.

NREMS substances	REMS substances	Wakefulness substances
IL1[1]	VIP	hypocretin
TNF	NO	CRH
GHRH	PRL	
NGF		
Adenosine		
Prostaglandin D,		

[1] Abbreviations: see Fig 1 legend and VIP, vasoactive intestinal polypeptide; PRL, prolactin

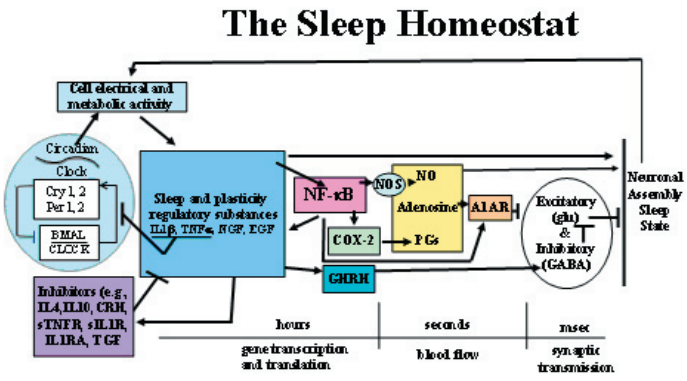

Figure 1. Cytokines such as IL1, TNF, nerve growth factor (NGF), epidermal growth factor (EGF),interleukin 4 (IL4), interleukin 10 (IL10), and associated soluble and membrane-bound receptors all form part of the sleep biochemical regulatory network. Cell activity affects levels of these substances. Within brain for example, ATP, co-released during neurotransmission, induces the release of the gliotransmitters IL1 and TNF from glia. These substances induce their own production and interact with multiple other substances via nuclear factor kappa B (NFkB) activation. These effects are associated with gene transcription and translation and take several hours. Down-stream events include well-known metabolic substances and regulators of the microcirculation such as NO, adenosine and prostaglandins. Neurotransmission, acting on an even faster time scale, is altered by these substances via actions on the production of receptors that alter postsynaptic neuron sensitivity such as AMPA and adenosine A1 receptors (A1AR). State oscillations within local networks occur as a result of this ultra-complex biochemical regulatory scheme (1,2). Abbreviations: NO, nitric oxide; PGs, prostaglandins; COX, cyclooxygenase; glu, glutamic acid; GABA, gamma amino butyric acid; CRH, corticotrophin releasing hormone; sTNFR, soluble TNF receptor;, sIL1R, soluble IL1 receptor; IL1RA, IL1 receptor antagonist; TGF, transforming growth factor beta; cry, cryptochrome; per, period; GHRH, growth hormone releasing hormone.

TNF-enhanced NREMS is most often accompanied by enhanced EEG delta power; this measure is indicative of sleep intensity and is used to model the S-process in the two process model of sleep regulation [3]. If TNF is injected into the pre-optic area (POA) of the anterior hypothalamus, a sleep regulatory circuit, it also enhances duration of NREMS and EEG delta power [9]. In contrast, if the TNF soluble receptor, an inhibitor of TNF, is injected into the POA NREMS duration is reduced. TNF can also enhance EEG delta power within local cortical circuits. Thus, unilateral injection of TNF onto the surface of the cortex results in ipsilateral enhancement of EEG delta power during NREMS but not during REMS or W. Conversely, the TNF soluble receptor unilaterally inhibits sleep loss-enhanced EEG delta power after cortical injections [10]. Similarly, if a TNF si RNA, a substance that decreases TNF mRNA stability, is unilaterally injected on to the cortex, it reduces TNF -immuno-reactivity and EEG delta power unilaterally only during NREMS [11].

TNF mRNA levels vary with sleep propensity; daylight levels are about 2 fold higher than nighttime levels. Similarly, TNF protein in hypothalamus and cortex is about 10 fold higher at the beginning of daylight hours compared to nighttime levels. TNF mRNA levels also increase in brain during sleep deprivation in rats [1]. In humans, plasma levels of TNF in normal healthy individuals co-vary with EEG delta power. As already mentioned, in a variety of pathologies that manifest in sleepiness or excess sleep, TNF plasma levels are enhanced. For instance, after influenza viral challenge in mice, hypothalamic TNF levels are enhanced at the time NREMS is also enhanced [12]. Similarly, giving humans endotoxin, a Gram-negative bacterial cell wall product, enhances TNF plasma levels and sleep [13]. Collectively the data discussed in this section clearly implicate TNF in physiological sleep regulation and in the sleep responses to sleep loss and pathological challenges.

Activity-dependent induction of TNF; a mechanism for how the brain keep track of past sleep-wake history

Neurotransmission is characterized by the co-release of ATP with neurotransmitters [14]. This extracellular ATP signals via purine receptors P2X and P2Y to release TNF, IL1 and brain-derived neurotrophic factor from glia [15-17]. Some of that extracellular ATP also rapidly degrades into adenosine and it in turn acts via P1 receptors. This is posited to be one of the rapid mechanisms involved in sleep induction (Figure 1). The ATP -released TNF also acts rapidly on cells to alter membrane potentials and these changes are also likely involved in sleep regulation [18]. Perhaps more important to sleep regulation are the long term (hours; Figure 1) actions of TNF. Thus TNF activates nuclear factor kappa B (NFkB), an enhancer element involved in the transcription of multiple substances involved in sleep regulation [19,20]. NFkB enhances transcription of the adenosine A1 receptor and the glu-R1 component of the glutamate AMPA receptor. Expression of these receptors alters the sensitivity of the neuron to adenosine, a hyperpolarizing inhibitory substance, and glutamate, a depolarizing excitatory substance. As a consequence, depending upon the relative number of each of these receptors, the sensitivity of the neuron to these ligands is altered. Such changes alter network input-output processes and are thought to be a key component in events such as memory formation and state changes (see below). This process is called scaling, up-scaling if there is a relative increase in the AMPA receptors and down-scaling if there is a relative increase in the adenosine A1 receptors (Figure 2). There is also direct evidence that TNF is involved in scaling [21].

If neurotransmission-associated ATP release is involved in a long-term mechanism for keeping track of past brain activity, then ATP agonists or antagonists should alter sleep. To our knowledge, such substances have not been characterized for their actions on sleep. Preliminary data from our laboratory suggest that intracerebroventricular administration of the ATP agonist, BZATP enhances NREMS while the ATP antagonist, OXATP, inhibits NREMS (De *et al*, unpublished). Further, other preliminary data indicate that P2 receptor mRNAs (both X7 and Y1) have a diurnal rhythm in brain and are altered by sleep loss, IL1 and TNF injection, and changes in ambient temperature (Taishi et al, unpublished). If such data are confirmed and extended, they will suggest that the mechanism by which the brain keeps long-term track of prior activity (states) involves neurotransmission-released ATP that in turn releases brain cytokines and their subsequent actions on receptor expression and consequent cell sensitivity to excitatory and inhibitory signals (Table 2). This also strongly suggests, because such a mechanism

is a local event involving autocrine and paracrine signaling within the neuronal assembly where the neurotransmission took place, that sleep is initiated locally and is, thus, fundamentally a local process [22,23].

There is also evidence suggesting that TNF immunoreactivity in neurons is dependent upon prior activity within the neurons. Thus within somatosensory cortical columns, if rat whiskers are repeatedly stimulated, the number of TNF immunoreactive pyramidal cells is enhanced within the column receiving afferent input from the stimulated whisker but not in adjacent columns [24]. Whether this TNF is produced within the neurons or is that TNF released from glia by ATP remains unknown. Nevertheless, the results clearly demonstrate the use-dependency of a SRS. In the next section, the ability of TNF to promote a local sleep-like state within cortical columns is described.

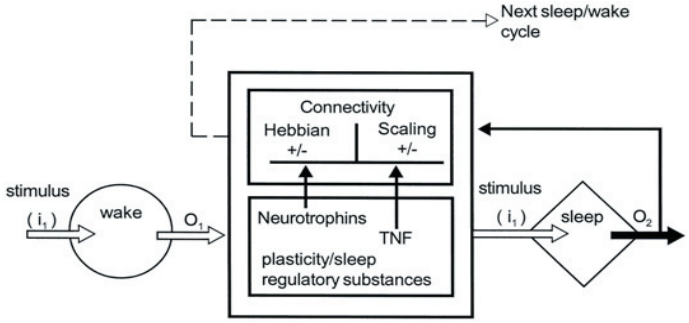

Figure 2. The cortical column as the basic unit of brain circuitry regulating sleep. A cortical column in the wake state exhibits a characteristic input-output relationship reflecting stimulus processing (left). With continued use, the metabolic condition of the cortical column changes, leading to enhanced production of sleep regulatory substances (SRSs) such as neurotrophins and tumor necrosis factor (TNF) within the column (center). The SRSs in turn alter the input-output relationship of the cortical column, modifying its stimulus response and rendering it effectively asleep (right). The use-dependent SRSs also sculpt neuronal and glial connectivity (center) by inducing synaptic plasticity (using Hebbian and scaling mechanisms), and this provides a possible function for the sleep state. In this conceptualization, sleep function is closely linked to local, use-dependent metabolism, and thus inseparable from sleep regulation. This paradigm provides a coherent physiological basis for sleep across species ranging from humans and other mammals to fruit flies and jelly fish.

Table 2. Mechanistic Hypothesis for Local Use-Dependent Sleep

Step 1: Metabolism and electrical activity link sleep regulatory substance (SRS) release and synthesis via ATP (sleep and metabolism are connected).
Step 2: SRS production is thus activity-dependent (sleep is homeostatically driven).
Step 3: SRSs act locally to alter receptive, hence electrical, properties of nearby neurons and thus alter input-output relationships of the network within which they are found (sleep is targeted to previously active networks).
Step 4: The altered input-output network relationships reflect a functional state change (sleep is local).
Step 5: Neuronal assembly sleep-like states synchronize with each other leading to organism state changes (organism sleep is a network emergent property)(see Roy et al, submitted, for a mathematical model).
Step 6: Sleep regulatory circuits coordinate the individual network (e.g. cortical columns) functional states into organism sleep architecture (sleep is adapted to the organism niche).
Step 7: SRSs act on multiple levels of the neural axis to promote sleep (sleep mechanisms are ubiquitous and evolutionarily ancient).

What sleeps? Sleep as a fundamental process of neuronal assemblies

A new paradigm for how the brain is organized to produce sleep posits that sleep regulation is fundamentally a local and use-dependent process[22,23,25]. This hypothesis is substantially different from the prevailing view that sleep and wake states are whole-brain, global phenomena imposed upon the brain by specialized sleep/wake regulatory circuits. The paradigm shift has considerable consequences for our understanding of sleep regulation, sleep pathologies and, ultimately, sleep function.

Although the involvement of specialized brain areas in sleep/wake regulation is well documented, there is no evidence that these specific networks are required or essential for the occurrence of sleep. Thus, for millions of stroke patients and thousands of animal brain-lesion studies, there is not a single report of a post-lesion survivor who failed to sleep. This suggests that no specific portion of brain is critically involved in the genesis of sleep *per se*. It also suggests that sleep is a fundamental property of any surviving group of neurons and that it is self-organizing.

It seems likely that brain tissue can express sleep locally, and that this may occur spontaneously, without top-down control [22,26]. Isolated cortical islands separated from thalamic input yet retaining their circulation display episodic slow waves in the EEG [27]. In whole brain EEG delta power is recognized as a signature property of NREMS. Further, as mentioned above, when the SRSs, TNF [10], or IL1 [28] or GHRH [29], are applied unilaterally to the cortex *in vivo*, they intensify EEG delta power during NREMS but not during REMS or W. This enhanced EEG delta power occurs only in the region where these SRSs are applied, not in the whole brain. These data strongly suggest that at least one sleep phenotype, EEG delta power, is a local property of brain tissue. In addition, some species such as birds and whales exhibit uni-hemispheric sleep; thereby directly demonstrating that sleep is a property of something less than the whole brain [30]. Clinical evidence also suggests that some patients can be awake and asleep simultaneously [31].

Evidence from the developmental and memory literatures suggests that local sleep is use-dependent. Thus a plethora of experimental interventions ranging from whisker stimulation in rats to unilateral somatosensory stimulation, arm immobilization, adroit learning paradigms in humans [1], and selective sensory deprivation of neonates [32,33] indicate that localized changes in sleep EEG delta power or blood flow [34] are enhanced if during prior waking the areas were disproportionately activated relative to other brain areas. Such findings support the idea that sleep is not only local in nature, but also that its expression is related to prior brain activity—i.e., that it is use-dependent.

Sleep can be independently expressed in units of brain as small as cortical columns. This may be the minimal component in brain capable of sleep [35]. Cortical columns are densely interconnected assemblies of neurons thought to be the basic unit of information processing. In these rat experiments, individual cortical columns (whisker barrels) exhibit responses called evoked response potentials that are a measure of input/output relationships. The evoked response potentials are greater during sleep than during wakefulness. Individual columns can show the characteristic responses during sleep while neighboring columns exhibit wake-like responses. Vice versa, individual cortical columns can display wake-like states while neighboring columns show sleep-like responses when the rat is functionally and behaviorally asleep. These data suggest a degree of cortical column local state autonomy although local column state usually corresponded with whole animal state. The local sleep state occurs more frequently when the cortical column at hand is stimulated more intensively thereby exhibiting a use-dependency of state. These results point to cortical columns as the basic unit of brain circuitry involved in sleep and sleep regulation. Finally, if TNF is applied locally to the cortex it enhances the amplitudes of evoked response potentials suggesting that it induces the local sleep-like states in cortical columns [36].

Cortical column state has an impact on overt behavior in rats [37]. In an experimental learning paradigm dependent upon sensory stimulation of a single whisker, when the corresponding whisker barrel was in the wake-like state, correct behavioral responses to whisker twitching were elicited. However, when the whisker barrel was in the sleep-like state, the rat made errors. In the prevailing top-down paradigm of sleep regulation, intentional action from the specialized sleep/wake regulatory brain circuits is required to initiate and terminate whole-organism sleep, raising unresolved questions as to how that purposeful action might itself be initiated. The new paradigm of local, use-dependent sleep regulation avoids such infinite regresses. It posits instead that local sleep is a direct consequence of prior local use and that whole-organism sleep is essentially a bottom-up, self-organizing, emergent property of the collective states of cortical columns throughout the brain [26]. In our theory, a role remains for specialized brain nuclei and pathways involved in whole-organism sleep regulation, e.g. coordination of cortical columns to synchronize into single whole-brain vigilance states and coordination of overall brain states with other physiological systems in the body including nitch-adapted circadian rhythms. However, sleep initiation resides in the individual cortical columns and other neural assemblies across the brain.

Implications for sleep function

The sleep regulatory mechanism presented results in the stabilization of cell sensitivity by changing receptor populations for inhibitory (adenosine) and excitatory (glutamate) molecules. This action occurs as a consequence of neuronal activity and is thus localized to the sites where activity-induced changes in synaptic efficacy and connectivity are occurring. Thus, SRSS, altered by activity, in turn alter expression of the receptors involved in plasticity. A comprehensive understanding of the molecular and genetic mechanisms of sleep remains incomplete. Nevertheless, our view of sleep mechanisms clearly link sleep and neural connectivity.

Our proposed sleep mechanism also provides insight into unconsciousness. We assume that within neuronal assemblies input during waking induces environmentally adaptive outputs. With prolonged activation of networks within the assemblies (excessive input) the mechanisms shown in Figures 1 and 2 and Table 2 would be activated and the consequent SRS release would induce a new output in response to the same input. The new output would likely be irrelevant to the environmentally-driven input. It would be maladaptive if allowed to manifest in cognitive or motor

real-time events because behavior would not be coordinated in real-time to environmental inputs. This creates an adaptive need to prevent the animal from behaving at such times. The local sleep mechanisms are as a consequence not only inseparable from the plasticity functions of sleep but they also provide the necessity for unconsciousness.

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