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Our Current Understanding of Theneurochemical Basis of Homeostatic Mechanisms that Regulate Sleep is Incomplete

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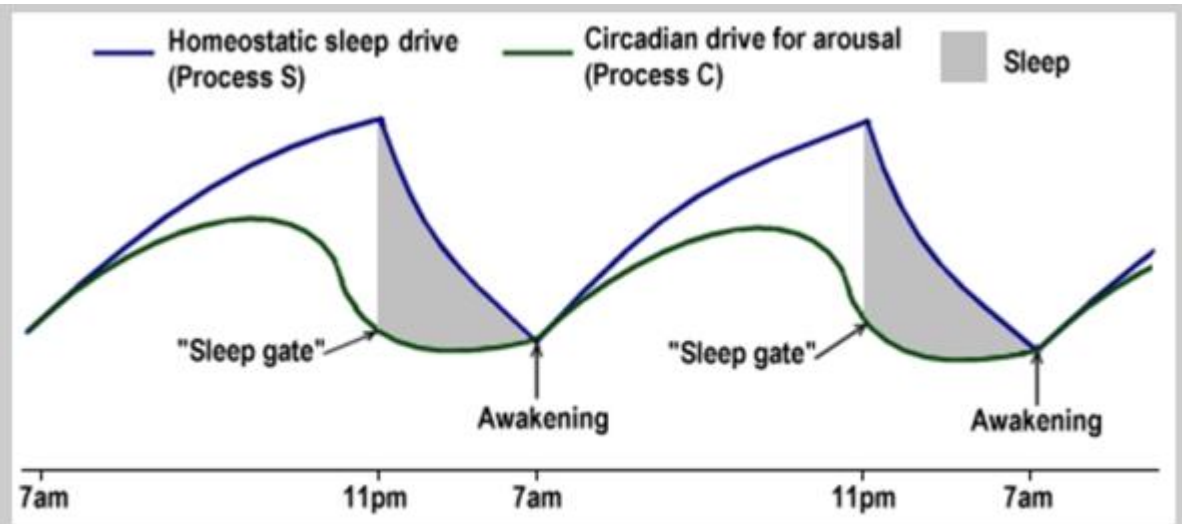
Abstract: ***The modern - day concept of 'sleep - wake regulation' was first proposed by Alexander Borbely in 1982. This two - process model proposes that there are primarily two mechanisms that control this cycle – 'process C' or circadian rhythm and 'process S' or homeostatic control. Numerous experiments in the past have enriched us with the knowledge of the neurochemical basis of the homeostatic regulation of sleep. The arousal systems are located mainly in the brainstem, posterior and lateral hypothalamus and basal forebrain. These networks utilize a number of neurotransmitters such as histamine, acetylcholine, serotonin, norepinephrine, dopamine, glutamate and orexin/hypocretin to promote wakefulness. The normal circadian cycle starts with the activation of SCN by sunlight. SCN then stimulates the LH and the TMN nucleus and through the neurotransmitters noradrenaline, serotonin and acetylcholine maintain wakefulness. During the day, the tonic activity of the SCN increases progressively and counteracts the homeostatic sleep drive till evening. By late evening, the pineal gland starts secreting melatonin. A second internal mechanism that promotes sleep results from the accumulation of inhibitory neurotransmitter adenosine which activates VLPO which in turn inhibits the wake - promoting nuclei and ensures sleep. Thus, the homeostatic regulation of sleep involves a very complex network of activities of different sleep substances, neurotransmitters and hormones on the various sleep and wake areas of the brain some of which have been discovered and described but there may be many more waiting to be discovered. Our present knowledge therefore is incomplete and lacks a complete grasp of the neurochemical basis of sleep.***

1. Introduction

Sleep, though an important aspect of health homeostasis, has been neglected for centuries. Though philosophers studied sleep about 2500 years ago, the modern - day concept of 'sleep - wake regulation' was proposed by Alexander Borbely only in 1982. This two - process model proposes that there are primarily two mechanisms that control this cycle –'process C' or circadian rhythm and 'process S' or homeostatic control which actually depend on how long we have been awake or asleep.[1]



Sleep-wake regulation: interaction between the homeostatic sleep drive (Process S) and the circadian drive for arousal (Process C) (image by Luke Mastin)

Figure 1

These processes in turn act on primarily the Basal forebrain, Hypothalamus, Midbrain and Hindbrain to produce cycles of REM and NREM during sleep.

Several studies have proven the role of VLPO nucleus and the Median preoptic area in the generation of sleep. [2, 3] As discussed in a review article by Ritche E. Brown, Radhika Basheer et al., 2012, [7]GABAergic nurons projecting from preopticarea to the Ascending reticular activating system (ARAS) have been shown to inhibit wakefulness and produce sleep. [4, 5] Furthermore, many studieshave also proposed aflip - flop switch model of transition between

sleep and wakefulness due to activation or inhibition of wake promoting or sleep inducing areas.

Jun Lu et al., 2006, also proposed a flip - flop switch for control of REM sleep present in the brainstem.[6]

The search for a single factor promoting sleep has been going on for several decades and researches in this field has led to the discovery of many substances that induce sleep. Factors promoting NREM sleep include Adenosine, NO, Cytokines such as TNF - α, IL 1, PGD2 and GHRH.[8] REM sleep is primarily regulated by Prolactin, NO, VIP.[1]

The neurotransmitters related to sleep induction have been shown to be GABA and Galanin which inhibit wakefulness - promoting neurotransmitters such as Histamine, Dopamine, Noradrenaline, Serotonin and Acetylcholine.[9]

Thus, the homeostatic regulation of sleep appears to involve a complex network of activities of sleep substances, neurotransmitters and hormones on the different sleep and wake areas of the brain some of which have been discovered and described but there maybe many more waiting to be discovered. Our present knowledge therefore lacks a complete grasp of the neurochemical basis of sleep.

Role of BDNF

Borbely in 1982 first stated that sleep is homeostatically regulated. He justified his statement by showing that both the duration and depth of sleep increases with periods of prior sleep deprivation. Faraguna, U., et al. 2008, hypothesized that SWA is high at sleep onset as there is widespread synaptic potentiation in cortical and subcortical areas in the preceeding waking period, leading to cortical expression of BDNF - Brain - Derived Neurotrophic Factor.[9] BDNF is a plasticity related gene, the secretion of which is strongly activity dependent. In their study on rats, they showed that more the rats explored, more was the expression of BDNF and the subsequent sleep SWA (slow wave activity). BDNF injections in unilateral cortex of awake rats showed that NREM - SWA was higher in the injected hemisphere as compared to the contralateral one. These effects were not seen in REM sleep. These facts prove that building up of sleep pressure results in increased BDNF and SWA during sleep.

They suggested that the possible cause of SWA by increased BDNF was probably synaptic potentiation in local circuits. In simulation studies, synaptic potentiation produced large amplitude single - cell slow oscillations, synchronization of which among connected cells may lead to large amplitudes. Further studies on living subjects is however necessary in this field.

Role of Adenosine and Orexin:

Mahesh M. Thakkar et al., 2003, studied the role of neuromodulator Adenosine in the homeostatic control of sleep.1[0]During wakefulness, metabolic activity leads to utilization of ATP. ATP breakdown causes accumulation of Adenosine, most marked in Basal forebrain. Several studies have shown that Adenosine levels increase during wakefulness and decrease following recovery sleep. Thakkar et al showed that after perfusion of Adenosine A1 antisense in the Basal forebrain, there was a reduction in NREM sleep and an increase in wakefulness along withsignificant reduction of delta - wave sleep.

In another study by Porkka Heinskanen et al., 2000, it was shown that though extracellular Adenosine levels rise in several areas during wakefulness, the rise is most marked in Basal forebrain and to some extent in cortex but does not occur in other brain regions. 'The site - specific accumulation of Adenosine during sleep deprivation suggests a differential regulation of Adenosine levels by as yet unidentified mechanisms', they postulated.

Adenosine has been shown to activate the sleep - promoting neurons in the Pre - optic area. Yuki C. Saito et al., 2013, showed using optogenetics that neurons from the Pre - optic area send projections to the orexinergic neurons in Lateral hypothalamus and inhibit them through their secretion of the inhibitory neurotransmitter GABA.[2]Using channel rhodopsin 2, they traced the pathway of the GABAergic neurons from the Preoptic area to the Lateral hypothalamus, where they synapse with the orexinergic neurons. They also optogenetically stimulated the GABAergic neurons and showed that their stimulation leads to inhibition of the orexin neurons. Their study also demonstrated that neurons from Preoptic area also project to other brain areas responsible for wakefulness. So, it is difficult to say the extent to which inhibition of orexinergic neurons contributes to NREM sleep time. However this is partly responsible for NREM sleep duration, and further studies are needed to show the effect of optogenetic / pharmacogenetic stimulation or inhibition of GABAergic neurons of preoptic area in animals lacking orexin, they suggested.

In a study in 2009 by Svetlana Postnova et al., a mathematical model for homeostatic regulation of sleep - wake cycles was used where the neuropeptide Orexin determined sleep regulation.[12]Constant firing of orexin neurons during wakefulness increased the synaptic efficacy and decreased the wake drive. As the orexin efficacy gradually decreases during the day, their excitatory input to Monoaminergic neurons decreases leading to their decreased output. Monoaminergic neurons which normally inhibit VLPO will gradually cease to do so. So, the VLPO neurons can increase their firing rate and inhibit the Lateral hypothalamus which will further inhibit the orexinergic neurons. Synaptic plasticity of orexin neurons was suggested to be the cause of cycling between wakefulness and sleep. However, like Adenosine there must be many more processes regulating the sleep - wake cycles still unknown.

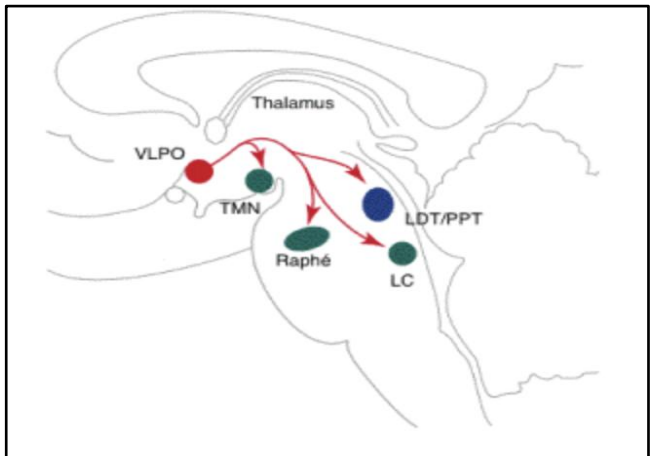


Figure 2: VLPO Projections

Role of PGD2:

T. Scammel, D. Gerashchenko et al., 1998, studied the role of PGD2 as a sleep promoting substance.[13]PGD2 was infused into the subarachnoid space just anterior to the preoptic area in rats which caused increased Fos staining associated with an increase in NREM sleep. Fos staining was increased in several sleep promoting areas and decreased in wake - active Tubo - mammary neurons. Probably PGD2 induced sleep by activating VLPO through the meningeal receptors, via paracrine release of Adenosine. Expression of Fos in other brain areas in their study could be related to sleep perse or due to the autonomic features associated with sleep. Further studies could definitely describe the role of these areas in sleep. Whether PGD2 could directly produce sleep or the exact mechanisms by which PGD2 activates VLPO remains to be established. A recent study by Zhi - Li - Huang et al., 2007, supported the role of PGD2 and Adenosine in regulation of sleep and wakefulness.[14]

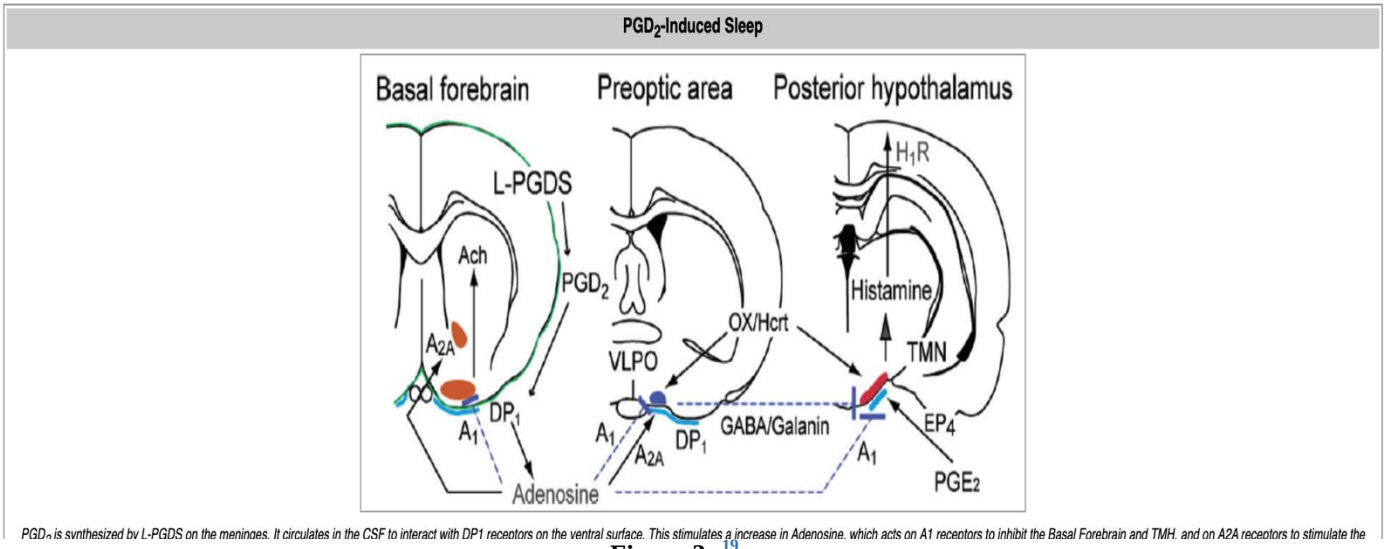


Figure 3: [19]

Role of Astrocytes:

In a very recent study by Dheeraj Pelluru et al., 2016, it was shown that there is a definite role of Astrocytes in generation of sleep.[15] In their experiment on wild mice, they optogenetically activated astrocytes in the posterior hypothalamus which increased both NREM and REM sleep. However, 'other brain areas need to be similarly manipulated to fully identify the role of astrocytes and neurons in regulating states of consciousness', they observed. Astrocytes are a major source of ATP and therefore also of extracellular Adenosine which is known to induce sleep. Genetically engineered mice with reduced Adenosine levels (expressing dominant negative SNARE within Astrocytes) do not have a sleep rebound after sleep deprivation as shown by Halassa et al., 2009, they noted.[16]Future studies in such mice could show that optogenetic stimulation of Astrocytes produced less Adenosine and blunted sleep response. Astrocytes are also known to release cytokines - IL1 and TNFα which are known to increase sleep. They expressed the desire to explore the role of Astrocyte released cytokines in affecting Orexin and Melanin concentrating hormone neurons.

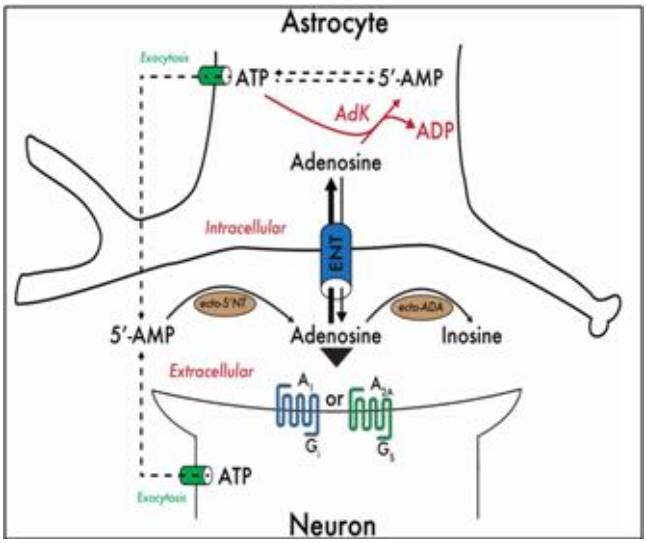


Figure 4: Control of the adenosine concentration by the metabolic state of astrocytes. Adenosine taken up by astrocytes is rapidly phosphorylated to AMP by adenosine kinase (AdK), an enzyme expressed predominantly in glia in the adult CNS. AdK effectively controls the intracellular adenosine concentration by catalyzing the transfer of a phosphate group from ATP to adenosine to produce ADP

and AMP. As a result, the rate of adenosine metabolism is reflected by the [ATP]/[ADP][AMP] ratio, linking the rate of adenosine metabolism to the metabolic state of the cell. Equilibrative nucleoside transporters (ENT) bi-directionally regulate the concentration of adenosine available to pre- and post-synaptic A_1Rs and A_2ARs. Other abbreviations used: 5′-NT, 5′-nucleotidase; ADA, adenosine deaminase.[20]

Role of NO:
NO is known to promote NREM sleep. NO produced by isoform of NO synthetase (iNOS) is responsible for this effect.8 However what stimulates iNOS during sleep deprivation is not known.

Genetic regulation:
Paul Franken et al., 2001, showed in a study on six inbred mouse strains that the rate at which SWS need accumulated as shown by delta power varied not only with duration of prior sleep deprivation but also genetically.1[7]They showed that the increased SWS need during wakefulness varies between genotypes, while its decreased need after sleep does not do so. As mentioned in their study, They identified two genomic regions that might affect the need for SWS. Chromosome 2 QTL containing genes encoding Brain - glycogen phosphorylase and two other genes encoding enzymes that regulate adenosine levels have a role. Additionally, genes for GHRH and somatostatin are also located in the same area. Aeschbach et al in 1996 compared delta power responses after sleep deprivation in human habitual long and short sleepers and arrived at a similar conclusion.1[8]QTL analysis in future may finally lead to identify genes responsible for homeostatic sleep regulation.

REM sleep mechanism:
The neuronal mechanism responsible for REM sleep is increased by firing of Glutamatergic and Cholenergicneurons of dorsolateral Pons. Projections from here to Basal forebrain produces EEG changes of sleep, while projections to medulla and spinal cord causes atonia of REM sleep. Jun Lu, David Sherman et al., 2006, proposed a flip - flop switch for control of REM sleep.6They proposed a brain - stem switch consisting of REM - off and REM - on areas in Meso - pontine tegmentum. Each side contains inhibitory GABAergic neurons and additionally the REM - on area also contains excitatory Glutamatergic neurons. Their model would provide a framework for further research in the field of sleep disorders, they observed.

2. Conclusion

Numerous experiments in the past few decades have enriched us with the knowledge of the neurochemical bases of the homeostatic sleep regulation. They range from lesion studies, Electrophysiology and cFos - staining to optogenetic studies and even genetic studies. Several studies have implicatedthe sleep - promoting effect of Adenosine via the A1and A2A receptor. The neurochemical pathway by which iNOS is stimulated during sleep deprivation to produce NREM is still unknown. Adenosine may have a role it has been suggested. Similarly, though the role of PGD2 in producing sleep has been shown in numerous studies, whether it has a role in directly inducing sleep or how it activates VLPO remains unanswered. Again, though it has been proven that GABAergic neurons projecting from PO area to the Lateral hypothalamus inhibit orexinergic neurons to decrease wakefulness, the effects of stimulating GABAergic neurons in animals lacking orexin remains to be seen, as such animals also have NREM sleep.

Genetic studies have also revealed the role of genes in homeostatic sleep regulation butfurther studies are necessary. Thus, though we now know that sleep is the result of inhibition of wake - promoting mechanisms by sleep substances like Adenosine, NO, PGD2, Cytokines like TNFα, IL1 etc., and the GABAergic neurons in preoptic area of the hypothalamus, the exact mechanism by which the brain senses the need for sleep, precisely which parts of the brain are involved or is it a network involving the whole brain remains unknown. The exact neurochemical control of this complex mechanism to this date remains a mystery. Until we solve this mystery we will not be able to address and treat the various sleep disorders.

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