4-19-2018

Mechanisms of Sleep and the Brain

Doungbagai Leo
Western Michigan University, doungbagaileo@gmail.com

Mechanisms of Sleep and the Brain

Doungbagai Leo

Western Michigan University

Abstract: One of the most important homeostatic functions of the brain and body is sleep. This literary review analyzes the genes that are involved in sleep: Clock, Bmal1, Period, and Cryptochrome. It also explains the role of melatonin, GABA, orexin, and melanopsin during sleep regulation. The genes and neurotransmitters influence the suprachiasmatic nuclei and the circadian rhythm that our brain, and every cell of our bodies, have connections to. These connection help to ensure the homeostatic function of our bodies. The analysis of sleep-like states in other animals may show an evolutionary connection of sleep from non-mammals to mammals, but may also not be connected to evolution at all because of lack of research and wrong lens analyses. Medication and meditation can provide alternatives to induce sleep and in the future, after more research is conducted, there may be better ways to induce sleep.

Introduction

As the bodies of animals became more complex, there came a need to maintain these bodies through homeostasis. The need to regulate temperature, heart rate, hunger, hormones, and more became an essential part of how an animal survived. One of the most essential parts of almost all walks of life is sleep or types of behaviors that are similar to sleep.

There are multiple stages of sleep: Awake, Non-REM1, Non-REM2, Non-REM3 and REM sleep. While awake, the electroencephalogram (EEG) reads alpha rhythms between 8-13 Hz. When sleep progresses to Non-REM1 the readings of the EEG are between 4-7 Hz. Non-REM2 is observed by using the EEG to find the production of sleep spindles and K complexes. Non-REM3 happens in the frontal region of the brain with EEG readings of 0.5-2 Hz with amplitudes of 75 microvolts. REM sleep is the observation of rapid eye movements (Őzşen, 2013). During the Non-REM sleep stages, also called slow wave sleep (SWS), there are bands read in the EEG that are called delta waves. During these delta waves, sleep spindle production is observed. It is thought that the sleep spindles encourage neural plasticity, the neurons ability to adapt to changes in the environment, and slow wave activity provides inputs to the suprachiasmatic nuclei about the current state of sleep homeostasis (Cajochen and Chellappa, 2010). During these stages heart rate decreases, breathing slows, body temperature decreases, and metabolism slows down. The sleep spindles that are produced during Non-REM2 may also play a role in the formation of thalamocortical networks by providing repetitive and synchronized signals (Lutter and Wakai, 2016). The K complexes that are also seen during Non-REM2 are waves characterized by a sharp negative wave followed by a positive wave. These waves have a duration of 0.5 seconds and 1.5 seconds with an amplitude larger than 75 μV (Ayappa, Parekh, Rapoport, and Selesnick, 2015). Though there are classifications of sleep that are widely accepted, the number of sleep stages are debated among scientists to be either 5 or 6 stages. For this paper we will

be looking at the 5 generally accepted classifications of sleep.

There are multiple processes or situations that may decrease one's ability to sleep. These include light from a television or cellphone, medications, illnesses, and environmental noise. Lights from a television or cellphone can disrupt one's ability to sleep because of the external light-dark cycles that are recognized by the retinal inputs that are sent to the suprachiasmatic nuclei that regulate the circadian rhythm (Cajochen and Chellappa, 2010). Medications may disrupt sleep depending on the impact they have on gamma-aminobutyric acid (GABA), melatonin, dopamine, and their receptors. An example of a medication that can have an impact on these neurotransmitters are benzodiazepines, which are prescribed for insomnia (Andenaes, Helseth, Misvaer, Smastuen, and Ribu, 2016). Illnesses impacting sleep include depression, Alzheimer's, insomnia, and certain cancers (Cajochen and Chellappa, 2010). Environmental noise also can impair a person's ability to sleep depending on the stage of sleep the person is in at the time. When more beta activity is observed on the EEG a person has a higher fragility to noise, compared to when slow wave sleep is observed (Mckinney, Scott, Thien, Orfeu, Jo, and Jeffrey, 2011).

Sleep is a homeostatic mechanism that ensures the survival of all walks of life. In the human brain, it is believed that synaptic plasticity is returned to a baseline during sleep (Cajochen and Chellappa, 2010). This may be because of the metabolites and neurotransmitters that are removed between synapses during sleep. When synapses are cleared of remaining neural waste during sleep, it allows neurons to function at full capacity when wakefulness is obtained.

As sleep continues to be one of the most integral parts of our lives, it would be of great importance to understand the reasons why it is important to our daily functions. This study is meant to use literary research in order to understand sleep mechanisms in our brain, sleep in other animals, and discuss alternatives to induce sleep.

Genes Involved in Sleep Regulation

Sleep is, in part, regulated by the genes that are expressed within our bodies. Genes of importance include Clock, BMAL1, Cryptochrome, and Period. These genes are not just turned on and off, but all work together to regulate sleep. The molecular relationship of Clock genes consists largely of Clock RNA and proteins that oscillate in a circadian style through positive and negative feedback loops of transcription and translation. The encoded core Clock proteins help to express the Clock and Bmal1 genes by binding to their transcription factors in DNA. This then creates the Clock-Bmal1 heterodimer that then translocates to the nucleus to induce the transcription of other Clock genes: Period and Cryptochrome. The Period genes include Per1, Per2, and Per3. The Cryptochrome genes include Cry1 and Cry2. Per3 induces the production of Per1/2 heterodimers that enter the nucleus to inhibit the production of Clock-Bmal1 mediated transcription. Per3 has also been seen to act as a marker of circadian gene expression and shows an entrained phase (synchronization), implying that Per3 may contribute to changes in sleep homeostasis. The autorepression of Cry1, Cry2, Per1, and Per2 creates a rhythm generating circuit that is found in most cell types, which regulate the circadian rhythms found in different cells within the body and the brain (Cajochen and Chellappa, 2010). These processes are meant to regulate the biological clocks in every cell of our body,

that then influence our circadian rhythm and sleep.

Neurotransmitters Involved with Sleep

Melatonin, GABA, and orexin have been found to be involved in sleep. Melatonin is shown to convey circadian-like influences on the suprachiasmatic nuclei (SCN) and throughout the body. It promotes sleep by lowering core body temperature and advancing the circadian rhythm. Melatonin is made in the pineal gland, and its precursor is L-tryptophan which is converted to 5-hydroxytryptophan, to serotonin, then acetylated to make aryl-alkylamino N-acetyltransferase (AANAT). AANAT is converted to melatonin by O-methyltransferase. AANAT is the rate limiting step for melatonin synthesis and is activated by the superior cervical ganglion. Norepinephrine acts as a beta-adrenergic receptor which triggers the production of cyclic adenosine monophosphate (cAMP) that promotes AANAT activity. The SCN also influences the reduction of norepinephrine that decreases the expression of AANAT and melatonin excretion. Melatonin has two receptors, MT_1 and MT_2. These receptors are guanine nucleotide G-protein coupled receptors that are expressed in the SCN but are also found in hypothalamic nuclei, retina, immune cells, and other peripheral organs. These receptors also inhibit the adenylate cyclase of cAMP to suppress the production of cAMP. This suppression depends on circadian time, duration, exposure to melatonin, and receptor dimerization and sensitivity. Melatonin not only helps to influence sleep by interacting with the SCN, but it reduces oxidative stress by scavenging for free radicals within the brain and body (Benarroch, 2008).

GABA is the primary neurotransmitter used by the SCN and has circadian influences on the SCN, to hypothalamic structures, and to the brainstem (Benarroch, 2008). There is evidence that shows that the $GABA_A$ receptor currents are changed depending on the amount of melatonin in a rat's SCN and hippocampus. The suppression of cAMP by melatonin increases the phosphorylation of $GABA_A$ receptor functions, which then influences the circadian couplings in the SCN (Brown, Cardinali, Niles, and Pandi-Perumal, 2010). Melatonin and the GABAnergic system may work together by reducing oxidative stress to help fight against sleep deprivation that is induced by anxiety (Choi, Joen, Kim, Kwon, Lee, and Shin, 2015).

Orexins (OX) are hormones that are believed to regulate physiological functions, like sleep, with connections to the posterior hypothalamus and interactions with monoamine (noradrenergic, serotonergic, dopaminergic, histaminergic, and cholinergic) systems. OX acts via G-protein coupled receptors and shows implied regulation of sleep and plays a differential role in neuronal pathways. These hormones also seem to be conserved in humans during physiological functions (Volkoff, 2012). OX also helps to maintain wakefulness and suppress REM sleep in mammals (Hayashi, Liu, and Miyazaki, 2017).

An Important Photopigment

An important photopigment that helps with the entrainment of circadian rhythm is melanopsin. This photopigment is also sensitive to blue light. Melanopsin is found in the inner retina which helps to send light stimuli to suprachiasmatic nuclei in the anterior hypothalamus via the retinohypothalmic tract. SCN have connections to the ventral lateral pre-optic area and this area receives direct input from melanopsin-containing photosensitive retinal

ganglion cells (pRGCs). These cells sense light pulses and sends those signals to suprachiasmatic nuclei. When melanopsin is absent, the transition from wakefulness to sleep is delayed (Archer and Dijk, 2009). It is important to note that the pRGCs that contain melanopsin do not depend on rods and cones to send light stimuli to SCN (Amir, Beaulé, Lamont, and Robinson, 2003).

Suprachiasmatic Nuclei (SCN) and Circadian Rhythms

The SCN is in the anterior hypothalamus. It functions as the biological clock by being the major pacemaker for various circadian rhythms. There is a specialized nonvisual retinohypothalmic tract that provides direct neuronal connections to the SCN from novel photoreceptors in retinal ganglion cells that measure the amount of external light. Before going to bed, melatonin receptors in the SCN suppress the firing of SCN neurons that are meant to promote wakefulness, so homeostatic sleep pressure is at the lowest levels (Cajochen and Chellappa, 2010). The SCN also resets daily because of its 24-hour cycle that humans live by. The resetting of the cycle depends on the light inputs from the retinohypothalmic tract and the amount of melatonin that is excreted during the night. The retinohypothalmic tract is made up of axons of intrinsically photosensitive retinal ganglion cells that express melanopsin and respond to the slow wavelength spectrum. The ability for the SCN to integrate environmental influences is from its intrahypothalamic connections that connect to the circadian system. Once the SCN is in sync with the external environment, it can help to regulate the sleep-wake cycle, hormonal secretions, thermoregulation, and more (Benarroch, 2008).

The SCN has processes that it follows to be the circadian pacemaker and regulate parts of the brain. As stated earlier, the SCN uses GABA as the primary neurotransmitter and there are two groups of GABA that it uses: one that synthesizes arginine vasopressin and the other that synthesizes vasoactive intestinal polypeptides. The SCN has prominent projections to the paraventricular nucleus (PVN) and the dorsomedial nucleus in the hypothalamus. The dorsomedial hypothalamus regulates circadian control, orexin/hypocretin protein systems, and wakefulness (Pandi-Perumal, Cardinali, and Chrousos, 2007). It gives stimulus to the dorsomedial nucleus by using the sub-paraventricular zone to stimulate sleep-wake cycles, corticosteroid secretions and feeding. It sends inhibitory responses to the PVN that then controls the sympathetic output of the pineal gland that controls the secretion of melatonin. Increased activity of the SCN is seen during light phases of photoperiods and decreases PVN activation that reduces norepinephrine secretions, AANAT activity, and melatonin secretions (Benarroch, 2008).

Now that the roles of the SCN, what it regulates, and what it controls is established, how does it tie into the entrainment and regulation of the circadian rhythm? Stated earlier, the SCN is the pacemaker of the circadian rhythm in the body, meaning it helps to influence the patterns that occur during our 24-hour cycle. The circadian rhythm is the biological timing system of our bodies that interacts with our environment to entrain the cells in our body and ensure mechanisms occur at the correct times. Our circadian rhythm can be disrupted, be delayed, advanced, or not shift at all by using an entraining agent. For humans it is the exposure of light during the first part of the night that delays our phase cycle, so light is the entraining agent for humans because of the retinohypothalmic tract that recognizes light pulses. Sleep has also been shown to help fight against cancer since the circadian

rhythm during sleep helps to regulate cytokines in our bodies, as cytokines are regulated during sleep and play a part in cell mitosis (Pandi-Perumal, Cardinali, and Chrousos, 2007). In one study involving zebrafish, they observed how circadian rhythms were entrained in the zebrafishes peripheral tissues using light. Zebrafish have a cell line called the Z3 which is the Clock gene for zebrafish. It was observed that the circadian gene expressions can be entrained using light because of the phototransduction and circadian oscillations observed in the peripheral tissues when the cells were exposed to light. This may also provide evidence to how the human body is entrained by light (Cahill, Cardone, Kaneko, and Sassone-Corsi, 2005).

The circadian rhythm is coupled with energetic cycles through transcriptional modulation by core Clock proteins and the sensitivity Clock genes have to redox states. In rats, redox reactions are used to rid the cell body of free radicals and in rats these reactions oscillate at the same time as the rat's circadian rhythm (Artinian, Coleman, Cox, Gillette, Govindaiah, et al., 2012). These redox reactions may also be similar in humans because of the homeostatic role that sleep has for clearing out neuronal waste between synapses.

Sleep in Different Animals

Sleep not only has a homeostatic role in humans, but it also plays a role in other animals. Sleep is also different in each animal, thus using sleep analysis for humans is insufficient for defining sleep in other animals. Scientists use these criteria to describe "sleep-like states" or behavioral quiescence in non-mammals: rhythmic activity, homeostasis, body posture, preferred resting locations, and decreased responsiveness to stimuli (Lyons and Michel, 2014).

Studies have been done on invertebrates regarding their sleep-like states. It has been observed that in the nematode, *Caenorhabditis elegans*, it has a quiescent behavioral state during a period called lethargus, which happens before each of its four moults. Lethargus is almost like sleep in that it has a constant temporal relationship with expressions in *C. elegans* an its larval stages. Their behavioral quiescence has a constant rhythm, reduced responsiveness to mechanical stimulation, and showed a state of "deep sleep" when mechanically stimulated for nine hours and then allowed to rest. This "deep sleep" observed in the nematode shows their body's attempt to return to homeostasis (Maycock, Pack, Raizen, Sundaram, Ta, and et al., 2008).

The annelid worm, *Platynereis dumerilii* was studied to find a connection between melatonin signaling in invertebrates to vertebrates. It was found that when they added luzindole, a melatonin antagonist, to the larvae their ciliary arrest time decreased while the added melatonin increased ciliary arrest time and created a sustained rhythm in the annelid worm (Nichols and Schippers, 2014). This suggests that melatonin has been conserved through evolution.

A sleep study was done on the crayfish, *Procambarus clarkia*, to test if sleep is a homeostatic mechanism for crayfish. They observed a sleep-like state with a specific posture, homeostasis, and reduced responsiveness. After continuous stimuli was put onto the crayfish, they observed longer sleep-like states to compensate for the continuous stimulation it had experienced. This sleep-like state is similar to slow wave sleep, and no REM stage is observed

(Bullock, Hernandez-Falcon, and Nguyen, 2004).

Researching invertebrates has provided us with basic understanding of mechanism of sleep, circadian rhythms, and neuronal plasticity (Lyons and Michel, 2014).

Sleep in non-mammalian vertebrate species is slightly different from mammalian sleep. Sometimes slow wave sleep and REM sleep are observed, sometimes they are not. Sometimes a more quiescence state is observed instead of something close to mammalian or avian sleep.

In reptiles, there are two sleep phases: quiet sleep and active sleep. During quiet sleep, it is thought that there is a replenishment of glycogen after its depletion during waking states and others suggest that is serves a role in biosynthesis and gene expression. Active sleep is proposed to play a role in maturation. Characteristics of sleep like states are also observed in reptiles (Herrel and Libourel, 2016). The sleep amplitudes in reptiles may depend on the temperatures of the environment. The desert iguana shows behavioral sleep plasticity in response to predation and will change their brain chemistry to stay awake if they sense a threat. Reptiles may also sleep with one or both eyes open or display slow wave sleep unihemispherically. The OX fibers in reptilian brains suggest a role for peptides in energy homeostasis and arousal in vertebrate groups (Volkoff, 2012).

Sleep in the amphibian, *Ambystoma tigrinium,* shows decreases in frequency and amplitude in brain wavelengths during long resting periods, clear neck atonia, and eye movement during sleep. The amphibian also shows slower brain activity while resting rather than when it is awake. (Herrel and Libourel, 2016).

Sleep in amphibians and reptiles are not yet understood, mostly because of the lack of studies about how they sleep. There isn't enough information to explain why certain down-scaling in neuronal pathways happen or why sleep amplitudes change in reptiles. These are gray areas and further studies are needed.

Birds are the only animals outside of mammals that have unequivocal SWS and REM sleep, but they are the only animals outside of mammals that displays both stages. The pigeons used in one study showed electrophysiological evidence for local sleep homeostasis in the avian brain. The changes in the avian brain also has similarities to sleep in the mammalian brain. These include slow wave activity in the visually deprived hyperpallium during recovery sleep that may show the change in synaptic strength, increase and decreases in slow wave activity locally in brain areas following use and disuse during wakefulness, more synchronous slow oscillations during subsequent sleep, and local increases in slow wave activity (Lesku, Martinez-Gonzalez, Ratenborg, Vyssotsk, and Wilzeck, 2011).

Sleep in mammals is very similar to avian sleep, but avian species do not display Non-REM sleep, which may be because of convergent evolution (Lyons and Michel, 2014). Non-REM sleep is involved in the secretion of growth hormones, synaptic plasticity, memory consolidation and clearance of brain metabolites. REM sleep regulates the cortical activity of other sleep stages and also helps with memory consolidation. In mice when REM sleep is inhibited by silencing GABAnergic neurons, mice did not consolidate what they learned after they had gone to sleep compared to mice that did consolidate what they learned when

REM sleep was not inhibited (Hayashi, Liu, and Miyazaki, 2017).

Sleep: Meditations and Medication

Age is another factor that can impair one's ability to sleep. This may be because of the loss of the cell's ability to remove metabolites and neurotransmitters between synapses. This can impair the ability of different mechanisms for sleep to occur in our cells. To combat this, meditation has been shown to provide positive effects for inducing sleep. Meditation is an approach that is non-pharmaceutical and older people are capable of understanding, learning, and practicing the meditation techniques. In one study, they provided self-relaxation meditation training in senior citizens and used a basic information questionnaire Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, and the Mini-Mental State Examination as ways to analyze the impact meditation had on sleep. The participants reported better sleep and were less disturbed by noises within the hospital after meditation (Kang, Sun, Wang, and Zeng, 2013). In another study conducted by the American Medical Association, they tested meditation on 24 random people ages 55 years or older. They found that mindfulness meditation improved sleep in older adults, better than structured programs that attempted to change bedtime routines (2015). The meditation technique Vipassana was also tested on multiple individuals from multiple different age groups. The groups were split into younger, middle, or older age. In the control group, the percentage of people that observed slow wave sleep were: younger = 11.29%, middle = 6.65%, and older = 3.94%. Meanwhile in the group that did meditation the numbers were: younger = 17.94%, middle = 11.3%, and older = 10.63%. In the control group the percentage of people that observed REM sleep were: younger = 21%, middle = 20%, and older = 18%. The group that did the meditation observed REM sleep at a higher rate: younger = 33%, middle = 30%, and older = 30% (Kutty, Nityananda, Pattanashetty, Sathiamma, Talakkad, et al., 2010). Meditation may provide a positive influence on sleep in many individuals and can be a safer and more productive alternative than medication.

Medication has also been a way to induce sleep within individuals that either have difficulty sleeping or psychiatric disorders. $GABA_A$ has been used to treat insomnia because $GABA_A$ promotes sleep in the preoptic hypothalamic area or in the brainstem that promotes Non-REM sleep. If the $GABA_A$ receptor gene increases channel currents, then it leads to increased sleep when mutated. GABAnergic neurons reduce sleep under genetic hyperpolarization (Hayashi, Liu, and Miyazaki, 2017). Insomnia is one of the most common sleep problems in Norway, as 6-15.5% of adult population meets formal diagnostic for it. Insomnia impacts sleep by lowering the threshold in which disturbances can be triggered. In a study with a sample size of 50,805 individuals, they observed that the number of medication users for sleep were 71.2% female and 12.5% of them had insomnia. Meanwhile in men, only 10.4% of them had insomnia. This may be interpreted as women have higher rates of insomnia than men. They also observed that more frequent doses of prescription medication were not associated with the reduction of insomnia. This evidence suggests that prescription medication only temporarily alleviates symptoms instead of curing the disease (Andenaes, Helseth, Misvaer, Smastuen, and Ribu, 2016).

Conclusion

Sleep is a homeostatic mechanism for all walks of life and the processes are complex

and not well understood. What is known is that when sleep deprivation is severe, it can lead to organismal death. Thus, the amount of time an organism sleeps, or stays in a sleep-like state, shows the homeostatic function of sleep. Neuronal plasticity is set to normal base-lines during sleep and reduction of free radicals within the body also decreases during sleep.

The characteristics of sleep in humans cannot be used to analyze sleep in other animals because other animals have different brain chemistries and live in different environments. Many of these studies involved comparing data of the human EEG to the EEG of crayfish, pigeons, or other animals. Many of these studies suggest that sleep has evolved through evolution but that may be because the analysis of sleep has been very human-centered. To understand sleep in an objective manner, I suggest stepping out of the human-centered lens and more into an individual species lens. This can allow for un-bias research for sleep in different species and may answer some unsolved questions about sleep in amphibians and reptiles.

One important factor that the studies of medication and meditation lacked was the impact that medication or meditation had on individuals each time they went to bed or if the individuals were able to function better the next day. Did the medication work every night? Did the meditation work every night? Was sleep achieved without disruption during the night? These questions remain unanswered. Observing people around us, we can see that medication does not always work every time, maybe increasing the doses can help but only so much of a medication can be safely given. Meditation requires a certain amount concentration and if someone is too stressed or anxious, then the relaxation effect may not be observed before they go to sleep.

Despite the lack of information regarding the daily effects of meditation, meditation can still be a healthy and positive alternative for sleep because of the relaxing influence it has on the brain and muscles of the body. It allows for self-awareness and one's autonomous control over how they will sleep. Of course, this does not ignore the changes in brain chemistry that comes with depression and insomnia, thus using low doses of medication with meditation may provide an alternative for inducing sleep also.

Sleep involves changes in brain chemistry and gene expression that can be altered with meditation or medication. Another alternative for the future, would be a device that can induce sleep, maybe using light and sound therapy with low brain wave stimulation. Though it sounds far-fetched, with more research into sleep, we can get to a point in which we could induce sleep daily without the side effects of medication and probability of meditation.

References

- American Medical Association. (2015). Meditation Promotes Better Sleep in Older Adults. *The Journal of the American Medical Association, 313(16),* 1609.
- Amir, S., Beaulé, C., Lamont, E.W., and Robinson, B. (2003). Melanopsin in the circadian timing system. *Journal of Molecular Neuroscience, 21(1),* 73-89.
- Andenaes, R., Helseth, S., Misvaer, N., Smastuen, M.C., Ribu, L. (2016). Psychosocial factors are strongly associated with insomnia in users and nonusers of prescribed sleep medication: evidence from the HUNT3 study. *Journal of Multidisciplinary Healthcare, 9,* 547–555.
- Archer, S.N., Dijk, D. (2009). Light, Sleep, and Circadian Rhythms: Together Again. *PLoS Biology, 7(6),* 1-4.
- Artininan, L., Coleman, T.P., Cox, C.L., Gillette, M.U., Govindaiah, G., Sweedler, J.V., Wang, T.A., Ye, X., Yu, Y.V.

(2012). Circadian Rhythm of Redox State Regulates Excitability in Suprachiasmatic Nucleus Neurons. *Science, 337*, 839-842.

- Ayappa, I., Parekh, A., Rapoport, D.M., Selesnick, I.W. (2015). Detection of K-complexes and sleep spindles (DETOKS) using sparse optimization. *Journal of Neuroscience Methods, 251,* 37-46.
- Benarroch, E. E. (2008). Suprachiasmatic nucleus and melatonin: Reciprocal interactions and clinical correlations. *CLINICAL IMPLICATIONS OF NEUROSCIENCE RESEARCH, 71,* 594-598.
- Bullock, T.H, Hernandez-Falcon, J., Nguyen, B. (2004). Slow Wave Sleep in Crayfish. *Proceedings of the National Academy of Sciences of the United States of America,101(32),* 11857-11861.
- Cahill, G., Cardone, L., Hirayama, J., Kaneko M., Sassone-Corsi, P. (2005). Genetic Approaches to Circadian Clocks: Analysis of Circadian Rhythms in Zebrafish. *METHODS IN ENZYMOLOGY, 393,* 186-204.
- Cajochen, C., Chellappa, S.L. (2010). The Circadian Clock and Homeostatic Hourglass: Two Timepieces Controlling Sleep and Wakefulness. *Protein Reviews 12,* 195-228.
- Cardinali D.P., Pandi-Perumal S.R., Niles L.P., Brown G.M. (2010) Melatonin and Sleep: Possible Involvement of GABAergic Mechanisms. In: Monti J., Pandi-Perumal S., Möhler H. (eds) *GABA and Sleep* (279-301). Springer, Basel.
- Choi, Y,Y., Han, S.H., Jeon, S.J., Kim, M. K., Kwon, K.J., Lee, E.J., Shin, C.Y. (2015). THE POTENTIAL ROLE OF MELATONIN ON SLEEP DEPRIVATION-INDUCED COGNITIVE IMPAIRMENTS: IMPLICATION OF FMRP ON COGNITIVE FUNCTION. *Neuroscience, 301,* 403–414.
- Herrel, A., Libourel, P.A. (2016). Sleep in amphibians and reptiles: a review and a preliminary analysis of evolutionary patterns. *Biological Reviews 91,* 833-866.
- Hayashi, Y., Liu, C., Miyazaki, S. (2017). Sleep in vertebrate and invertebrate animals, and insights into the function and evolution of sleep. *Neuroscience Research 118,* 3-12.
- Kang, J., Sun, J., Wang, P., Zeng H. (2013). Self-relaxation training can improve sleep quality and cognitive functions in the older: a one-year randomised controlled trial. *Journal of Clinical Nursing, 22,* 1270–1280.
- Kutty, B.M., Nityananda, P., Pattanashetty, R., Sathiamma, S., Talakkad, S., Trichur, R. (2010). Practitioners of vipassana meditation exhibit enhanced slow wave sleep and REM sleep states across different age groups. *Sleep and Biological Rhythms, 8,* 34–41.
- Lesku, J.A., Martinez-Gonzalez, D., Rattenborg, N.C., Vyssotsk, A.L., Wilzeck, C. (2011). Local sleep homeostasis in the avian brain: convergence of sleep function in mammals and birds*? The Royal Society, 278,* 2419–2428.
- Lutter, W.J., Wakai, R.T. (2016). Slow rhythms and sleep spindles in early infancy. *Neuroscience Letters, 630,* 164-168.
- Lyons, L.C., Michel, M. (2014). Unraveling the complexities of circadian and sleep interactions with memory formation through invertebrate research. *Frontiers in Systems Neuroscience, 8(133),* 1-13.
- Maycock, M.H., Pack, A.I., Raizen, D.M., Sundaram, M.V., Ta, U.D., You, Y., Zimmerman, J.E. (2008). Lethargus is a Caenorhabditis elegans sleep-like state. *Nature, 451(06535*), 569-574.
- McKinney, Scott M., Thien Thanh Dang-Vu, Orfeu M. Buxton, Jo M. Solet, and Jeffrey M.Ellenbogen. (2011). Covert waking brain activity reveals instantaneous sleep depth. *PLo SONE, 6*(3): e17351.
- Nichols, S.A., Schippers, K.J. (2014). Deep, Dark Secrets of Melatonin in Animal Evolution. *Cell, 159,* 9-11.
- Őzşen, S. (2013). Classification of sleep stages using class-dependent sequential feature selection and artificial neural network. *Springer, 23,* 1239-1250.
- Pandi-Perumal, S.R.,. Cardinali, D.P., Chrousos, G.P. (2007). Neuroimmunology of Sleep. New York: Springer.
- Volkoff, H. (2012). Chapter 17: Sleep and Orexins in Nonmammalian Vertebrates. *Elsevier 89,* 315-339.