The potential role of DNA methylation in sleep deprivation dependent disorders

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Abstract

Insufficient sleep is associated with numerous negative effects as cognitive impairment, mental disorders and cardiovascular diseases. To fully understand how sleep deprivation (SD) can lead to these impairments and conditions, it is essential to know the underlying mechanisms. Despite the consensus about the importance of sleep as a restorative mechanism, the processes behind this are still to be fully described. Here, I hypothesize that reduced clearance of waste products in the brain and epigenetic changes to DNA form the basis of the long-lasting consequences of sleep deprivation. In peripheral tissue, lymph vessels are used to return excess metabolites and proteins into the blood stream. The brain does not have lymph vessels and returns its metabolites to the blood circulation via the glymphatic system. Astrocytic aquaporin-4 water channels (AQP4) conduct water through the cell membrane, stimulating convective flow. The increased flow increases cerebrospinal fluid (CSF) and interstitial fluid (ISF) interactions. This process is extensively reduced (>60%) during wakefulness thus leading to increased metabolite and waste product levels in the brain. These waste products can cause long-lasting DNA damage and are shown to be involved with neurodegenerative diseases such as Alzheimer. Additionally, sleep deprivation has shown to alter DNA methylation patterns of the genome. DNA methylation is an epigenetic process in which a methyl (CH3) group is added to the DNA, altering the function of genes. This is a stable modification that can suppress gene expression over a lifespan. Previous studies have showed that DNA methylation is a way to adapt to (stressful) environmental conditions by altering gene expression. These adaptations may also occur in situations of prolonged sleep deprivation, though at the cost of increased chance for diseases and cognitive impairments. Hypothesizing the mechanisms behind these negative consequences can lead to a better understanding of the underlying processes involved in sleep deprivation.

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Introduction

Sleep is essential to human life and has both physiologic and behavioral components.(Ednick et al., 2009) Altered consciousness and inhibited sensory activity are key aspects of sleep seen in many organisms including humans(Campbell & Toblew, 1984). Besides these behavioral aspects, other processes like energy metabolism synaptic plasticity and neuroprotection differ between sleep- and awake-state(Kreutzmann, Havekes, Abel, & Meerlo, 2015; Tononi & Cirelli, 2014). Ever since the recorded history, sleep has been a fascinating topic generating many questions and theories. This interest in sleep finds it origin with the ancient Greek and Romans and is still a major field of study in the biology(Barbera, 2007). Despite all these years of effort, the mechanisms that underlie sleep and the effects of sleep deprivation (SD) are yet to be fully described.

There are many processes that differ between sleeping and being awake. Most of them are anabolic processes in which new proteins, lipids or other molecules required during the day are synthesized during sleep. In this way the body builds up muscular, immune and nervous systems. Other systems that work in an alternative way during day and night are metabolite clearance from the interstitial space and the production of melatonin(Xie et al., 2013)(Aukland & Reed, 1993)(Schomerus & Korf, 2005). It is interesting to see how these processes are influenced by SD and what consequences SD has on factors like performance and mood. In addition to that, many studies showed that SD as well as disruption of the humane 24 hour (circadian) rhythm are associated with many diseases like diabetes(Nagorny & Lyssenko, 2012) and several mental disorders like schizophrenia and Major Depression Disorder(Lamont et al., 2007). Having in mind these diseases, which can arise even much later in life then the occurrence of the SD, it is interesting to search for the mechanisms behind leading to these diseases.

A hypothesis which will be proposed here is: the basis of these phenotypical effects lays in the genetics. Epigenetics is a new field within the biology which focusses on the interaction of DNA with its environment. In the epigenetics two types of mechanisms are described: histone modification DNA methylation. Due to histone modifications and DNA methylation, the expression of genes can be altered or even switched off. One example of DNA methylation can be found in eukaryote totipotent and pluripotent stem cells. These cells differentiate into multiple different cell types by switching certain genes off and activating others through DNA methylation (Wolffe, 1999). Apart from cell differentiation, histone modification and DNA methylation sleep can have effects on the expression of genes not involved with cell differentiation. For instance important circadian genes (BMAL1,Clock,CRY1 and PER1) which are involved in regulating rhythmic gene expression (Cedernaes et al., 2016). SD does not only alter clock gene expression, SD also alters expression levels of genes which are involved with sleep homeostasis, oxidative stress and metabolism (Moller-Levet et al., 2013).

In addition to the previous hypothesis, metabolite clearance may contribute to the long-term negative consequences of SD. Metabolite clearance in the interstitial space in the brain is a process which is prominently active during the night, with an activity that is 60% to 70% higher during sleep then during wakefulness(Xie et al., 2013). This removal of toxins and metabolites may be one of the restorative functions of sleep and is affected heavily by SD. These metabolites like β-amyloid can accumulate, cause DNA damage and can even lead to development of neurodegenerative diseases like Alzheimer(Ho, Ortiz, Rogers, & Shea, 2002). altogether this process can induce long-lasting DNA damage and together with epigenetic changes possibly contributing to the negative consequences of SD.

Finally the role of melatonin will be discussed. There is a lot of research done searching for the role of melatonin in relation with sleep and the timing of sleep. New data however shows another function of melatonin. It is found to be a highly effective free radical scavenger(Tan et al., 2007). Besides in the brain, melatonin is found in the gut and in the skin, both organs that are in close contact with the environment and need to cope with hostile elements. To protect this tissue from hostile elements melatonin is used as reactive oxygen scavenger (ROS). In contrast with the skin and gut, the brain does not have to be protected from external elements, but rather from its own waste products. Due to a high metabolism and oxygen consumption large numbers of free radicals and other waste products like metabolites are released. Melatonin can be used to prevent these waste products to cause damage. SD coincides with a bright environment which reduces melatonin production(Schomerus & Korf, 2005) and therefore SD can decrease the availability of melatonin as (ROS). This decreased availability of melatonin can aggravate the irreversible effects DNA damage caused by these waste products.

In this article, different mechanisms that may underlie the long term consequences of SD will be described and analyzed. The role of epigenetics will be coupled with basal mechanisms that may interact with this process. Additionally, processes that work via different pathways will be taken into account and related to the effects of SD. The outcomes of this article may add some extra knowledge to the molecular pathways involved in these long-term negative consequences SD.

Why sleep?

Sleep is a state which is seen across all animal species and is therefore thought to be essential to animal life including humans(Campbell & Toblew ', 1984).Characteristics of sleep involve both physiological and behavioral components like altered consciousness and inhibited sensory activity (Ednick et al., 2009). Sleep comes with a reduced responsiveness to external stimuli; during this period organisms are exposed to potential danger. This increased risk of predation as well as other threats during sleep are dangerous and therefore sleep should also have beneficial effects that may provide the essential needs of animals(Campbell & Toblew ', 1984). Besides these behavioral aspects, other processes like energy metabolism, synaptic plasticity, and neuroprotection differ between sleep- and awake-state(Massart et al., 2014). Tononi and Cirelli (2003) proposed a number of hypotheses describing the essential functions of sleep. One aspect of this theory is synaptic downscaling. Synaptic downscaling can increase the signal to noise ratio by reducing the signal of "noise" neurons to a level below which they can be detected. Neurons which send a stronger signal will remain, while "noise" neurons that send contrasting weaker signals are made silent or ineffective. This process decreases competition between neurons and counteracts synaptic potentiation. Besides that, synaptic downscaling is important for the homeostasis of synaptic weight. The process of downscaling neurons creates brain space for plastic modifications and prevents saturation.

Sleep is essential to brain function is a commonly accepted fact in the scientific world. Many processes involved with brain functioning and cognition are already shown to be associated with sleep. However there is no consensus about the specific mechanisms which are involved(Kreutzmann et al., 2015). Nonetheless the involvement of preserving neuronal plasticity and homeostatic recovery seems to correspond between studies. Besides that many studies already showed that a lack of sleep has an opposing effect on cognitive functioning, leading to impaired concentration and memory as well as to a prolonged reaction time(Kreutzmann et al., 2015)(Xie et al., 2013). Effects of chronic SD include mental

disorders like schizophrenia and Major Depression Disorder(Lamont et al., 2007). In addition, chronic SD is associated with elevated risk for hypertension and an increase in mortality(Wolk et al., 2005). Countless studies have shown the consequences of acute as well as chronic SD. Yet the mechanisms that form the basis for the development of these diseases are yet to be fully described. In the following chapters a number of possible mechanisms will be proposed.

Metabolite clearance

Sleep has a critical role ensuring metabolic homeostasis. In peripheral tissue, lymph vessels are used to return excess metabolites and proteins to the blood circulation. From there it travels to the liver where it is degraded(Aukland & Reed, 1993). The brain however, has no lymph vessels and has to remove metabolites and toxic products in different ways from their neurons. In figure 1, the glymphatic system is described in which cerebrospinal fluid (CSF) interacts with interstitial fluid (ISF) removing interstitial proteins like β-amyloid(Xie et al., 2013). Influx of cerebrospinal fluid (CSF) locates around the arteries in para-arterial side of the brain, here it interacts with interstitial fluid (ISF) removing interstitial proteins which leave the brain via the veins. This glymphatic system is based around the astrocytic aquaporin-4 water channels (AQP4) which conduct water through the cell membrane (Aukland & Reed, 1993). Deletion of this AQP4 water channels showed a decreased CSF influx and a reduction of interstitial proteins of 65%(Iliff et al., 2012). Xie et al. (2013) found similar results in both sleeping and anesthetized mice; in both cases, the clearance of proteins was significantly reduced in wakefulness, when comparing to sleeping or anesthetized mice. The increased clearance of intestinal proteins during sleep and anesthesia adds up to the restorative theory of sleep in which the neurons in the brain are freed from the degradation products which are formed during the day.

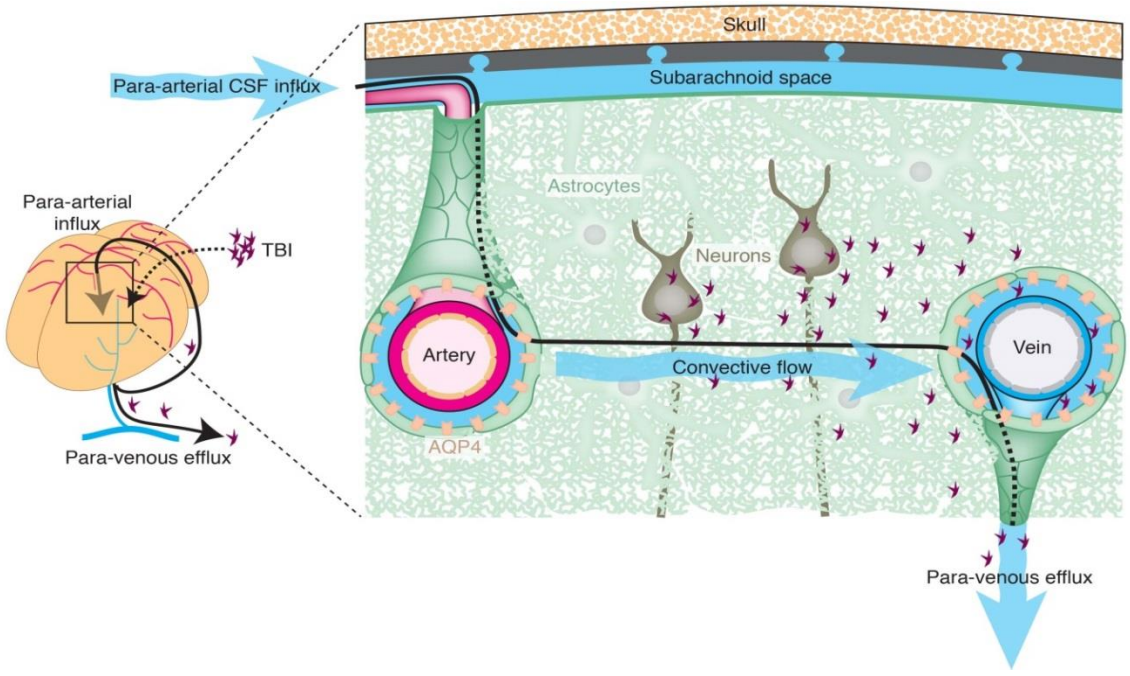

Figure 1 The glymphatic system. Obtained from: http://jonlieffmd.com/blog/immune-t-cells-are-critical-for-cognitive-function

In a situation of SD**,** the glymphatic does not work optimal, leaving waste products in the brain. These waste products, e.g. β-amyloid peptides, which can cause DNA damage and mitochondrial dysfunction leading to cellular damage Accumulation of β-amyloid peptides is found to be involved with neurodegenerative diseases like Alzheimer. β-amyloid together with hyper phosphorylated tau work in an additive way causing progression of the disease. In which β-amyloid is shown to progress the disease partly by inducing oxidative damage(Mao & Reddy, 2011). Not only β-amyloid induces DNA damage, also Homocysteine can promote this DNA damage. Homocysteine is a neurotoxic amino acid which can extracellularly accumulate in the brain and is correlated with numerous neurological disorders, cardiovascular diseases and Alzheimer(Ho et al., 2002). Thus the possible effects of SD in reduced clearance of these macronutrients in the CSF does not only cause cellular complications in the form of mitochondrial dysfunction, but also irreversible DNA damage.

Another process which is influenced by SD and may relate to DNA changes is the production of melatonin. Melatonin is known as the hormone of the night and is secreted by the pineal gland under the control of the main circadian pacemaker; the suprachiasmatic nucleus (SCN). The SCN generates a circadian rhythm of approximately 24 hours and entrains the body to external stimuli like the photoperiod. In mammals the SCN receives information from retinal photoreceptors about the ambient light conditions. At night, the SCN signals the pineal gland to start producing and secreting melatonin(Schomerus & Korf, 2005). The production of melatonin is suppressed mainly by light but also in minor ways through physical activity and food intake(Lewy et al., 1980; Lynch, Eng, & Wurtman, 1973)(Wyatt et al.,1999). In a lot of SD cases, at least one of the suppressing factors of melatonin production is involved, leading to reduced melatonin availability. As mentioned before, melatonin is not only involved in the timing of body functions but melatonin is also an excellent antioxidant and free radical scavenger(Tan et al., 2007). Mistimed suppression of melatonin production due to SD thus not only has a negative effect on endogenous timing, but also on preventing cellular and DNA damage.

Both the reduced removal of intestinal toxins as well as the decreased production against ROS may be an explanation for the increased chance on diseases and psychiatric disorders(Lamont et al., 2010). On top of that, it underlines the importance of sleep as a restorative process.

Sleep deprivation during development

Sleep deprivation can have extensive negative influences on mood, learning and performance the following day, but can also have consequences throughout one's life. One way to study these effects is using patients with Obstructive sleep apnea (OSA). OSA is a condition in which the upper airway is either partly or completely obstructed during sleep. When these apneas happen the breathing stops for a brief moment leading to reduced blood oxygen saturation and reduced sleep quality(Daroff, 1991). People tend to have decreased sleep intensity and can wake up during these episodes of airway obstruction. Therefore these patients are an interesting group for studying the effects of chronic SD on the development of neurophysiological components and cognitive functioning. The presence of SD however coincides with OSA itself which may be the cause of these deficits. Halbower et al. (2006) analyzed the data of a group of 31 children (19 OSA, 12 control) and found that the children with severe OSA had an significantly reduced IQ along with potentially damaged frontal cortex and hippocampus. Similar data was found in a survey about sleeping behavior of children between 5 and 7 years old. Children with a

history of sleeping disorders like OSA scored worse on measurements regarding depression symptoms, social problems and attention. Besides that, cognitive abilities were lower for the sleeping disorder group then the controls (O'Brien et al., 2004), illustrating the importance of sufficient sleep on cognitive functioning. Especially during the first year of human life, babies spend over half of their day sleeping. In a review article of Ednick et al. (2009) several studies on the effect of sleep on the development and maturation of the central nervous system (CNS) are summarized which are showed in table 1. The overall conclusion is that decreased sleep efficiency during the first year of life correlated with a lower Mental Developmental Index at 10 month old infants. Besides that, a positive association was found between Rapid Eye Movement (REM) activity and mental development. Adding evidence to the importance of (REM) sleep on the maturation of the CNS. Together these studies add more evidence to the importance of sleep during this critical period in development for the normal development of the CNS.

Despite outcomes of SD the processes behind these developmental deficits are still mostly unknown. Another question which is still unanswered is why certain brain regions like the hippocampus are more vulnerable to SD then other brain regions(Havekes, Vecsey, & Abel, 2012). One indication of possible mechanisms behind these long term negative consequences of sleep loss are long lasting changes in gene expression(Cedernaes et al., 2016; Massart et al., 2014)(Liu & Chung, 2015).

Gene expression

Gene expression is a process in which the nucleotide sequence of genes is transcribed into functional gene products (RNA). This RNA encodes for molecules like proteins and lipids which are necessary for normal body functioning and mental health. The gene expression of the human transcriptome varies over the day and numerous genes are expressed with a circadian rhythmicity. In a situation of SD, the body has different needs and the circadian expression rhythm of certain genes is not the desirable rhythm of RNA synthesis for normal body functioning. Moller-Levet et al., (2013) found that acute SD in humans disrupts the circadian regulation of the transcriptome by altering gene expression of multiple "clock" genes including PER1, PER2, CRY2 and CLOCK. Also gene expression of genes involved in oxidative stress, sleep homeostasis and metabolism were altered; all processes that have an important relation with sleep. These changes in gene expression can be the result of a diverse range of processes. Promoters and enhancer proteins for instance, can trigger the start of transcription of genes. Other (epigenetic) factors can both promote and suppress gene expression. These epigenetic processes are DNA methylation or histone modifications. These epigenetic modifications can be stable, suppressing gene expression as long as a lifetime. These modifications are preserved during cell divisions and are therefore an interesting area to study the long-term effects of SD. Especially considering, changes in gene expression are known to be involved with numerous diseases(Moller-Levet et al., 2013)(Liu & Chung, 2015).

Table 1—Key Aspects and Results of Studies Correlating Sleep and Mental Development

Author	N	Mode of Sleep Assessment	Timing of Sleep Assessment	Mode of Development Assessment	Timing of Development Assessment	Correlations Between Sleep Characteristics and Mental Development
1. Beckwith et al.[75]	53 premature infants	EEG	40 weeks CA	Gesell Developmental Scale	4, 9 months	*tracé alternant* during QS: 4 months, $r = 0.32$ 9 months, $r = 0.33$ No significant association with QS
2. Scher et al.[76]	16 preterm infants + 16 term infants	EEG	1-2 weeks after birth, then monthly up to term age	BSID	12 months, 24 months	***No. of arousals, $r^2 = 0.6382$, Adj $r^2 = 0.6053$
3. Anders et al.[77]	24 premature infants	Time-lapse video recording	2, 4, 8, 20, 24, 36, 52 weeks' CA	BSID	24, 52 weeks' CA	Holding Time Index: 24 weeks conceptional age (CA), $F = 5.9$ Longest Sleep Period: 52 weeks CA, $r = -0.40$, $F = 7.6$
4. Scher[78]	50 healthy infants	Questionnaire, actigraphy	10 months	BSID	10 months	Sleep efficiency, $r = 0.30$ % of activity/minute of sleep, $r = -0.30$ # awakenings > 5 minutes, $r = -0.37$
5. Becker et al.[79]	29 healthy infants	EEG	6 months	BSID	1 year	REM Storms: Group 1, $r = -0.65$ Group 2, $r = -0.88$
6. Arditi-Babchuk et al.[80]	81 premature infants	Observation	34 weeks CA	BSID	6 months	Prediction of MDI at 6 months REM ($\beta = 29$, $F = 5.96$) No association between REM storms and MDI
7. Freudigman et al.[81]	36 healthy newborns	Motility Monitoring System	Postnatal days 1 & 2	BSID	6 months	Longest sleep period, $r = -0.42$ Sleep-wake transition time, $r = 0.36$
8. Montgomery-Downs et al.[82]	35 healthy infants	Overnight polysomnogram	8 months	BSID	8 months	Snore-related arousals, $r = -0.43$, $R^2 = 0.18$
9. Gertner et al.[83]	34 premature infants	Actigraphy	32 & 36 weeks CA	BSID	6 months	36 weeks CA: Total night mean activity level, $r = 0.373$ Total sleep %, $r = -0.349$ Total night sleep %, $r = -0.405$ No significant associations with sleep assessed at 32 weeks' CA No significant association with QS
10. Borghese et al.[88]	49 premature infants	Motility Monitoring System	36 weeks CA & 6 months	BSID	6 months	Active Sleep: 36 weeks CA, $r = -0.44$ 6 months, $r = 0.34$ Cyclicity: 36 weeks CA, $r = -0.40$ 6 months, $r = 0.43$ No significant association with QS
11. Whitney et al.[87]	100 premature infants	Motility Monitoring System	Weekly for 1st 5 weeks of life	BSID	1 year, then biannually until age 3	Neurodevelopmental group: > Time in waking active < Time in Active Sleep and QS < Active sleep bout length < Longest sleep period < State stability score
12. Shibagaki *et al.*[89]	27 infants*	Overnight polysomnogram	4 months-1 year	Tsumori-Inage Questionnaire	4 months-1 year	Developmental quotient, $r = 0.88$, $R^2 = 0.78$
13. Scher *et al.*[90]	100 infants**	Questionnaire	4-6 months, 10-12 months	Harris Infant Neuromotor Test	4-6 months, 10-12 months	Similar sleep patterns in infants with and without risks for developmental delays

*infants with developmental disabilities; **infants were divided into 4 groups based on risk for developmental delay ; ***unadjusted for prematurity

Key Aspects and Results of Studies Correlating Sleep and Mental Development. Study characteristics including number of infants, modes of sleep and development assessment, and timing of sleep and development assessments are provided. Significant correlations are provided between various sleep characteristics and mental development scores for each study as well.

Abbreviations: EEG = electroencephalogram; CA = conceptional age; BSID = Bayley Scale of Infant Development; QS = quiet sleep; r = correlation coefficient; R^2 = squared multiple correlation; F = variance ratio

Table 1 Correlations between sleep during the first year of infancy and mental development(Ednick et al., 2009)

Epigenetics

This relative new field, studies (heritable) changes in gene expression that occur without changes in DNA sequence. These changes can switch genes on and off, depending on the place the modifications occur. There are two main categories of epigenetic changes namely: histone modifications and DNA methylation. Histone arginine and lysine methylation have attracted many researchers to study the role of these modifications on both the active and repressive effects on chromatin function. In addition, the enzymes that catalyze the methylation were identified and are still one of the main subjects in epigenetic research. (Robert J. Klose and Yi Zhang, 2007).

In contrast with histone modifications which are mainly dynamic and vary throughout the day, DNA methylation is a much more stable and irreversible process. In humans about 70 percent of the DNA is methylated which can be attributed to normal development and cell proliferation. Embryonic stem cell differentiation requires a constant repression of genes which are not needed in specific tissue or cell types. Therefore the genome-wide landscape of DNA methylation is the basis for cell functioning and the transcriptional potential of a cell(Wolffe, 1999). An example of DNA methylation is X chromosome silencing in which genes located at the X chromosome are partly silenced in males. These genes which are repressed are silenced through the methylation of the promoter regions. This specific DNA methylation is continual over life however decreases slightly with age (Migeon, Beur, & Axelman, 1989; Samollow, Robinson, Ford, & Vandeberg, 1995).

The continuous changes in gene expression due to methylation can also directly be involved with diseases including Beckwith-Wiedemann syndrome and Angelman syndrome. In these syndromes one of the maternal alleles is methylated which leads to expression of just one of the maternal alleles causing overgrowth, increased risk of cancer and developmental disabilities.

In this paper the focus will lie on DNA methylation since it is a more stable process than histone modification and it may have a part in the negative long term effects of sleep deprivation. Furthermore DNA methylation is known to be an adaptive process for coping with (changing) environmental conditions. These epigenetic adaptations are already found in rats, that are challenged during infancy with either an environment with limited nutrition or low maternal care (Chmurzynska, 2010; G Weaver, Szyf, & Meaney, 2002). These rats had altered DNA methylation patterns of genes involved with stress responds such as glucocorticoid receptors. Hence this may suggest that these alterations prepare the stress responds to an environment with increased stress.

DNA Methylation

DNA methlation is a process in which a methyl (CH3) group is added to the DNA. It can occur in two of the DNA's nucleotides namely, adenine and cytosine. Adenine methylation is however an exlusively prokaryoot process which does not occur in eukaryotes. Nonetheless, cytosine methylation does appear in mammales and attaches a methyl group to the 5th position of cytosine, creating 5-methylcytosine (5mC) as shown in figure 2. This process mainly takes place in CpG sites which are regions in the DNA in which a cytosine is followed by a guanine (Robert J. Klose and Yi Zhang, 2007). DNA methylation works through two mechanisms: *De novo* DNA methylation and maintenance methylation. *De novo* DNA methylation is conducted by two main enzymes: DNA methyltransferase (DNMT) 3a and 3b which

establish methylation patterns during development and are important in the diffirentiation of stemm cells. Maintenance methylation is conducted by DNA methyltransferase1 (DNMT1) and is a process that preserves the methylation state during DNA replication (Chen & Riggs, 2011a). Demethylation can occur passively due to failure of DNMT1 but also actively, however the enzymes involved are not identified in mammels yet.

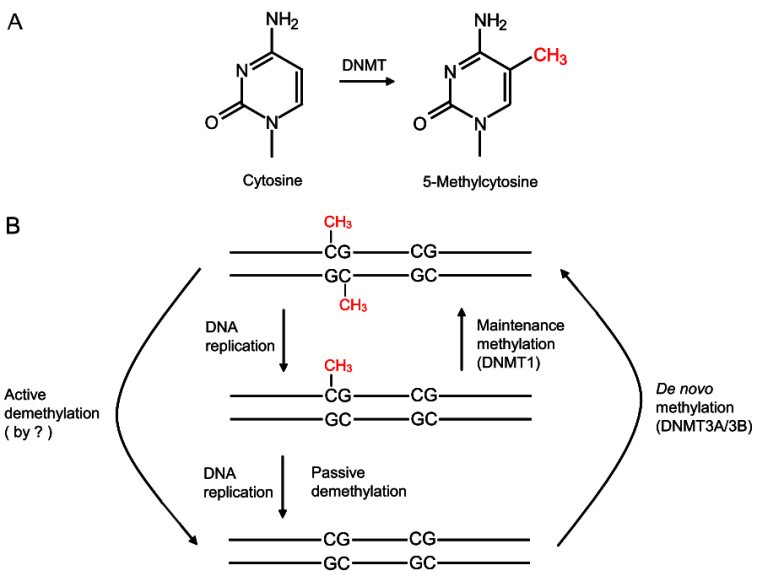

Figure 2 DNA methylation and demethylation in mammals(Chen & Riggs, 2011b)

The CpG sites where the methylation takes place are not evenly distributed across the genome, but can be clustered in so called CpG islands (CGIs); areas in the genome with high prevalence of CpG regions. New methods using bisulphite treated DNA (which detects 5mC and hydroxymethyl-Cytosine) enables genome-wide studies to the methylation of the human genome (Krueger & Andrews, 2011). This method has shown, that in vertebrates over half of the genes contain these CGIs (Jones, 2012a). Depending on the locations of these islands, methylation of the cytosines has contrasting functions. 5mC at the promotor region of genes can suppress or even silences genes, while methylation of CGIs located at the gene body enhances gene expression (Jones, 2012b). This however is not equal throughout all tissues and was not found in dentate granule neurons (Guo et al., 2011a). Research in these neurons also showed that synchronous neuronal activation induced *de novo* methylation and rapid demethylation in 1.4% of the genes observed (Guo et al., 2011b). This implies that DNA methylation is a more dynamic process then previously described and could be more important in neuronal plasticity then creating long-lasting modifications in the nucleus.

DNA methylation after sleep deprivation

DNA methylation may have multiple causes, among them environmental stress, persistent neuronal activity, and cell differentiation(Azzi et al., 2014; Cedar & Bergman, 2009). The relation between SD and DNA methylation however are less well studied in the context of the molecular pathways. SD did show alterations on long term-memory forming and consolidation which are processes that require DNA methylation (Kreutzmann et al., 2015). But no causal relation was found, connecting SD with methylation. In contrast with a group from Sweden who did found a (causal) connection. They looked at the methylation pattern and gene expression of key clock genes after one night of SD in humans. They found that In peripheral tissue, methylation was increased in promotor regions of CRY1 and PER1, showing also a decreased expression of these genes (Cedernaes et al., 2016). Likewise Massart et al. (2014) found broad changes in the methylation landscape of the cerebral cortex, suggesting that DNA

methylation is the basis of changes in gene expression after SD. In the same study also upregulation of Dnmt3a1 and Dnmt3a2 was found, which are enzymes responsible for *de novo* methylation. Additionally they found changes in the methylation of genes related with gene expression, neurotransmission, synaptic assembly and cell signaling which are processes involved with both regulating gene expression as well as synaptic plasticity.

Not only sleep deprivation, but also changes in day length may have influence on the methylation pattern of the DNA. In an experiment by Azzi et al. (2014) mice were kept under a day length of either 22-hour or 24-hour. This revealed alterations in methylation patterns at promotor regions in the SCN, leading to changes in gene expression. These changes could be reversed after extended (over 2 weeks) re-entrainment to a 24-hour cycle. This could indicate that DNA methylation serves as an adaptive process, adjusting to environmental conditions such as chronic sleep deprivation or altered day length. This suggests that DNA methylation may be a beneficial coping strategy.

Discussion

In conclusion: sleep is essential for normal functioning, which is shown especially after SD. Chronic SD can lead to reduced cognition and performance. Besides that it can trigger the development of diseases and disorders. The processes that underlie to the development of this long-term negative consequences are not yet described. Therefore DNA methylation and damage due to reduced clearance of waste products are proposed as mechanisms that may form the basis of these consequences. Additionally, reduced availability of melatonin may contribute to the damage induced by ROS caused by waste products.

Analysis of the possible mechanisms responsible for the long-term consequences of SD showed that not one process should account for all the outcomes of SD. It is more likely that DNA methylation and reduced clearance work in an additive way, complementing their effects. Furthermore only few studies are done observing the relation between chronic sleep deprivation and DNA methylation. therefore, prolonged observation of these methylation patterns should be done to examine to what extend DNA methylation and demethylation are dynamic processes. When studying these parameters it is interesting to explore the differences between young and mature animals including humans, since DNA methylation is also seen as a mechanism to preserve genotypic plasticity. A mechanism especially important for infants to cope with changing environmental conditions. This adaptive process may imply, that long-term DNA methylation of genes is a better alternative than a transcriptome which is not adjusted to its surroundings. Therefore DNA methylation might be seen as a process to prevent further (future) damage at the cost of constant repression of genes. For instance, DNA methylation after chronic SD may change gene expression in such a way that it enhances sleep intensity. Likewise it may increase metabolite clearance speed in a way that the body is adapting to an environment where sufficient sleep is not possible.

Anyhow before this can be tested, knowledge about *de novo* methylation and the consolidation of these methylated regions needs to be gained. Because the lack of this knowledge, the involvement of methylation in the long-term consequences of SD stays but a hypothesis. Along with that, histone modifications could play a greater role in stable regulating of gene repression than originally thought. Due to findings of Cedar & Bergman (2009) which showed that there is a “crosstalk” between histones and DNA methyltransferases. These findings suggest that the two processes may be dependent on one

another in a way that DNA methylation cannot take place without histone modifications. In addition to that histone modifications are shown to be involved in mechanisms repairing DNA after damage. Histones can help with either the repairing DNA or they can silence damaged genes with DNA methylation, as seen in several cancers (Friedl, Mazurek, & Seiler, 2012; Tjeertes, Miller, & Jackson, 2009). Therefore DNA methylation should perhaps not be seen as an active process adapting an individual to its environment, but rather as a mechanism which copes with DNA damage instantly. This coping strategy may be the cause for the development of future diseases.

Another way to explain impaired (cognitive) performance after SD is proposed by Vyazovskiy et al. (2012). In rats he found that groups of neurons can go in so called "local sleep" during prolonged wakefulness. Thus reducing the availability of neurons leading to impaired performance. This indicates that negative consequences after SD might not be attributed to molecular changes alone, but rather to systematic changes in brain organization as well.

Altogether there is still a broad field open for studying the basal mechanisms that account for the long-lasting negative or adaptive consequences of SD. When studying the mechanisms, it is important to look at the effects of chronical sleep deprivation on the methylation patterns as well as the consolidation of these. In addition to that it may be interesting to see, whether the assumption that DNA methylation acts as an adaptive process is true. This by studying if SD adapted animals can cope better with SD then non adapted animals. Although science achieved a lot since the first theories of the ancient Greeks about the purpose of sleep, new questions about the mechanisms underlying sleep remain unanswered.

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