THE EFFECTS OF SLEEP DEPRIVATION ON SPATIAL MEMORY, DREAM AND HYPERPHOSPHORYLATED TAU PROTEINS EXPRESSION IN RATS

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by

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ACD	: Acute Circadian Disruption
AChE	: acetylcholinesterase
Aβ	: amyloid β peptide
ANOVA	: Analysis of variance
APS	: Ammonium persulphate
ARASC	: Animal research and service centre
BCA	: bicinchoninic acid
BDNF	: brain-derived neurotrophic factor
BF	: basal forebrain
BSA	: bovine serum albumin
BWg	: body weight gain
CA	: connus ammonis
CA1	: connus ammonis region 1
CA2	: connus ammonis region 2
CA3	: connus ammonis region 3
Ca^{2+}	: calcium
CaM	: calmodulin
cAMP	: cyclic adenosine monophosphate
Cavβ3	: a beta intracellular subunit of the voltage-gated calcium channel
CC	: Cage control
CCD	: Control Circadian Disruption
CD	: Circadian disruption
Cdk5	: cyclin-dependent kinas
CHCD	: Chronic circadian disruption
CNS	: central nervous system
CRE	: cAMP-responsive element
CREB	: cyclic AMP response element binding protein
DAB	: diaminobenzidine
DG	: dentate gyrus
dH_2O	: deionised water

DNA : deoxyribonucleic acid

DPN : DREAM positive neuron

DRE : downstream response element

DREAM : downstream regulatory element antagonist modulator

DT : distance travelled

EC : entorhinal cortex

EEG : electroencephalogram

EF1 : first EF hand

EF2 : second EF hand

EF3 : third EF hand

EF4 : fourth EF hand

EL : escape latency

ELISA : enzyme linked immunosorbent assay

EMG : electromyogram

ER : endoplasmic reticulum

ETC : electron transport chain

Fc : food consumption

GA : Golgi apparatus

GABA : gamma-aminobutyric acid

GlcNAc : β-N-acetylglucosamine

Glu : glutamate

GLUT : glucose transport system

H_2O_2 : hydrogen peroxide

HCl : hydrochloric acid

HRP : horseradish peroxidase

IDV : integrated density values

IgG : immunoglobulin G

IHC : immunohistochemistry

K^+ : potassium

KChIPs : voltage-gated potassium (Kv) channels-interacting proteins

Kv : voltage-gated potassium

LD : Light: Day

LTD : long term depression

LTP : long term potentiation

MAPK : mitogen- activated protein kinase

mPRF : medial pontine reticular formation

mRNA : messenger RNA

MS : medial septum

MWM : Morris water maze

n : number

Na^{2+} : sodium

Na_2HPO_4 : disodium hydrogen phosphate

$Na_2HPO_4.7H_2O$: sodium phosphate heptahydrate

NaCl : sodium chloride

$NaH_2PO_4.H_2O$: sodium dihydrogen phosphate dehydrate

$NaHCO_3$: sodium bicarbonate

NaOH : sodium hydroxide

NCS : Neuronal Calcium Sensor

NCX2 : sodium-calcium exchange 2

NEPER : nuclear protein extraction

NGS : normal goat serum

NMDA : N-methyl-D-aspartate

NPY : neuropeptide Y

NREM : non-rapid eye movement

NT : neurotrophin

OD : optical density

PAGE : polyacrylamide gel electrophoresis

PB : phosphate buffer

PBS : phosphate buffered saline

PC : Pedestal control

PFA : paraformaldehyde

PHF : Paired helical filament

PKA : protein kinase A

PKC : protein kinase C

POMC	: pro-opiomelanocortin
PPN	: Positive PHF Neuron
PS	: paradoxical sleep
PSD-95	: post synaptic dendrite -95
PT	: probe test
R	: rapid eye movement group
REM	: Rapid Eye Movement
REMsd	: REM sleep deprivation
RER	: rough endoplasmic reticulum
RN	: rapid eye movement and nicotine treated group
RNA	: ribonucleic acid
rpm	: revolutions per minutes
S.E.M	: standard error mean
SDS	: sodium dodecyl sulphate
SDS-PAGE	: sodium dodecyl sulphate polyacrylamide gel
SPSS	: statistical package of social sciences software
SWS	: slow wave sleep
TBS	: tris buffer saline
TBS-T20	: tris buffer saline-Tween 20
TBS-TX	: tris buffer saline- Triton X-100
TEM	: transmission electron microscopy
TEMED	: N,N,N’N’-tetramethylenediamine
TF	: Transcription factor
TPN	: Tau Positive Neuron
Zt	: Zeitgeber

KESAN KEKURANGAN TIDUR PADA MEMORI, PENGHASILAN PROTEIN DREAM DAN FOSFORILASI BERLEBIHAN PROTEIN TAU KE ATAS TIKUS

ABSTRAK

Ritma sirkadian dikawal selia oleh biologi dalaman yang mengawal aktiviti fisiologi yang berbeza termasuk kitaran sel, suhu badan dan metabolisma badan. Pemantapan memori berlaku semasa fasa tidur yang dinamakan '*rapid eye movement'* (REM), jadi apabila ritma sirkadian terganggu dan juga fasa REM ini terganggu, memori akan turut terjejas. Protein yang dinamakan *Downstream regulatory element antagonist modulator* (DREAM) didapati memberi kesan kepada pembelajaran dan memori. Proses fosforilasi Tau protein yang berlebihan akan menyebabkan ketidakstabilan pada fungsi sel neuron dan akan mempengaruhi proses pembelajaran. Kajian ini bertujuan untuk mengkaji kesan gangguan tidur dan sirkadian (*circadian disruption* - CD) pada memori. Seratus empat puluh empat ekor tikus *Sprague Dawley* telah digunakan dalam kajian ini. Dalam eksperimen CD, gangguan sirkadian akut (*acute circadian disruption* - ACD) telah dilakukan dengan mengawal ritma sirkadian pada tikus ACD tiga jam awal dari hari sebelumnya. Gangguan ini telah dilakukan selama 6 hari diikuti oleh 10 hari rehat (*re-entrainment*). Proses yang sama telah diulang selama empat kali dalam kajian gangguan sirkadian kronik (*chronic circadian disruption* - CHCD). Pada kumpulan lain, pengurangan tidur fasa REM telah laksanakan dengan meletakkan mereka pada platfom kecil di dalam tangki berisi air (kumpulan REMsd). Kumpulan kawalan REMsd (PC) telah dimasukkan ke dalam keadaan yang sama seperti kumpulan REMsd kecuali mereka telah diletakkan di atas

platform yang lebih luas di mana mereka dapat tidur dan berehat dengan baik. Kumpulan kawalan dalam sangkar (CC) telah dimasukkan ke dalam sangkar biasa. Ia diikuti oleh ujian *Morris water maze* (MWM) untuk menilai kebolehan memori berkenaan ruang (*spatial*), platform tersembunyi (*probe*) dan ruang secara berbalik (*reverse spatial*). Kemudian, otak tikus bahagian hipokampus telah dikeluarkan dan nukleus serta sitoplasma neuron diekstrak untuk protein DREAM, Tau dan Tau terfosforilasi dianalisa dengan kaedah pewarnaan *immunohistochemistry* (IHC) dan *Western Blot* (WB). Ujian MWM mengukur tempoh masa untuk tikus berenang untuk mencari platform (EL) dari setiap kumpulan. Tempoh perbezaan EL selama lima hari didapati tidak berbeza secara signifikan ($p > 0.05$) di antara semua kumpulan dalam eksperimen REMsd tetapi didapati signifikan lebih lama pada kumpulan ACD dalam eksperimen CD. Ujian *probe* untuk MWM menunjukkan perbezaan yang tidak signifikan ($p > 0.05$) di antara kumpulan dalam eksperimen REMsd dan CD. Ujian mengukur memori secara berbalik juga menunjukkan perbezaan yang tidak signifikan di antara semua kumpulan ($p > 0.05$) dalam eksperimen REMsd dan CD. Ekspresi protein DREAM menunjukkan tiada perbezaan secara statistik direkodkan di antara kumpulan dalam eksperimen REMsd dan CD. Protein Tau dan Tau terfosfolirasi didapati berbeza secara statistik di antara kumpulan REMsd dan CC ($p < 0.05$) dan kumpulan ACD dan CCD ($p < 0.01$) dalam eksperimen REMsd dan CD, tetapi tidak signifikan berbeza terhadap ekspresi protein Tau yang terfosfolirasi berlebihan. Manakala untuk analisis WB menunjukkan tahap protein DREAM secara relatifnya tidak signifikan berbeza di antara kumpulan dalam eksperimen REMsd dan CD. Purata relatif Tau terfosforilasi berlebihan adalah tidak mempunyai perbezaan statistik yang signifikan di antara kumpulan dalam eksperimen REMsd dan CD. Kesimpulannya, protokol yang digunakan dalam kajian ini tidak berjaya menghasilkan perubahan dari

segi tingkah-laku otak dan tahap ekspresi protein DREAM dan fosforilasi berlebihan protein Tau.

THE EFFECTS OF SLEEP DEPRIVATION ON SPATIAL MEMORY, DREAM AND HYPERPHOSPHORYLATED TAU PROTEINS EXPRESSION IN RATS

ABSTRACT

Circadian rhythm is regulated by an endogenous biological regulator controlling various physiological activities, including cell cycle, body temperature, and metabolism. Some consolidation of memory occurs during Rapid Eye Movement (REM) sleep, so when the circadian system breaks down and REM sleep depressed, the memory will be affected. Downstream regulatory modulator antagonist (DREAM) proteins were discovered to affects learning and memory, while Tau protein hyperphosphorylation indicates some degree of neuronal instability influencing learning processes. This study aimed to examine the effects of sleep and circadian disruption (CD) on memory. One hundred and fourty four Sprague Dawley rats were used in this study. In the CD experiment, acute circadian disruption (ACD) was induced by putting the rats on a three-hour phase advance method for 6 days followed by 10 days of re-entrainment. This cycle was induced four times in a chronic circadian disruption (CHCD) study. On the other hand, REM sleep deprivation was induced by putting the rats on a small platform in a water tank (REMsd group). The REMsd control group (PC) was placed in the same condition as the REMsd group except that they were put in a broader platform to sleep and relax (PC group). The free-moving control group (CC) was placed in normal propylene cages (CC group). It was followed by the Morris water maze (MWM) task to assess spatial, probe test, and reverse spatial test. Then, the rat's brain from the hippocampal region was removed. The neuron's nucleus and cytoplasm were extracted for DREAM, normal Tau, and phosphorylated

Tau protein expression analyses by Immunohistochemistry and Western Blot (WB) analysis. In MWM test, the mean daily escape latency was measured for all rats from each group. The differences of EL between the groups were not statistically significant ($p > 0.05$) when compared between all groups in REM and CD experimental groups. The probe test showed no statistically significant ($p>0.05$) difference between the groups in the REMsd and CD experiment. The reverse spatial trial showed no significant difference between all groups ($p>0.05$) in the REMsd and CD experiment. DREAM protein expression was not statistically significant difference between groups in the REMsd and CD experiment. Tau and phosphorylated Tau protein was found to be significantly different when compared between REMsd and CC groups ($p<0.05$) and ACD and CCD group in the REMsd and CD experiment, but not significant difference for hyperphosphorylated Tau protein expression. WB analysis showed that the mean relative DREAM protein level was not statistically significant between all groups in the REMsd and CD experiment groups. The mean relative hyperphosphorylated Tau difference also was found not statistically significant between groups in the REMsd and CD experiments. In conclusion, the current study protocol failed to adequately elicited significant changes in neurobehavioral and level of the DREAM and hyperphosphorylated Tau protein expression.

CHAPTER 1
INTRODUCTION

Learning and memory consolidation and sleep-dependent are one of the areas of interest in neuroscience. Many studies have shown the importance of sleep in memory consolidation, which is a crucial part of the learning process (Stickgold and Walker, 2005, Maquet, 2001, Smith, 1995). Rapid Eye Movement sleep stage (REM) is related to non-declarative memory consolidation, which is essential in learning a new skill related to the procedure (Rasch and Born, 2013).

Lifestyle has now been increasingly stressful; failure to manage it may be affecting the quality and quantity of sleep needed. Furthermore, the arrival of portable touchscreen devices which are widely used in online social interaction and essential in fitting the needs of excellent work performance might worsen the situation of sleep quality. Studies have shown the sleep duration has become less, and the onset of sleep has become longer due to difficulties of falling asleep, as observed among high school and college students. These are the stages where learning is an essential part of their life (Peach et al., 2015).

Active learning induces neuronal excitation in the brain cortex. During sleep, neuronal excitation will be transferred to the inner part of the brain. This process will firmly consolidate the memory (Ngo and Staresina, 2017, Marshall and Born, 2007, Stickgold and Walker, 2005; BuzsÁk, 1998). This explains briefly and superficially how sleep in learning and memory are interrelated.

1.1 Rationale of The Study

Therefore, the current study aimed to explore the effects of REM sleep deprivation and circadian rhythm disruption on learning and memory by investigating the behavioural analysis and brain proteins measurement.

1.2 General Objective

The general objective is to measure the expression and level of DREAM and hyperphosphorylated Tau protein expression in REM sleep deprivation and circadian rhythm disruption in Sprague Dawley rats.

1.3 Specific Objectives

1.3.1 To evaluate the effects of REM sleep deprivation and circadian rhythm disruption on spatial memory in Sprague Dawley rats.

1.3.2 To measure DREAM and Hyperphosphorylated Tau proteins expression in Sprague Dawley rat's hippocampus after REM sleep deprivation and circadian rhythm disruption experiment.

1.3.3 To measure the level of DREAM/calsenilin and Hyperphosphorylated Tau protein expression in Sprague Dawley rat's hippocampus after REM sleep deprivation and circadian rhythm disruption experiment.

CHAPTER 2
LITERATURE REVIEW

Throughout all living organisms, sleep is a part of the most crucial cycle of their daily life. It carries a long list of physiological functions, and some of them are still elusive. Sleep deprivation affects many aspects of all living organisms. Sleep is not merely just a state of unconsciousness. It is defined as the behaviour of temporary perceptual disengagement from the external environment. However, from the inside, it involves a very complex physiological process and changes which carry all the essential function for human body revitalization before the next wake up cycle (Carskadon, 2011).

2.1 Sleep

A few physiological changes are associated with sleep. Physically, sleep dissociates a person from the environment, and it is associated with loss of consciousness. During sleep, the muscular hypotonic leads the postural changes to a recumbent position. Eye closure inhibits light entrance to the retina, thus cascading the sensory insensitivity in which its threshold gets higher as the sleep moves from arousal to deeper sleep stages (Datta, 2010).

2.1.1 Stages of Sleep, REM Sleep Versus NREM Sleep

Sleep can be divided into two stages: non-rapid eye movement (NREM, pronounced Non-REM) and rapid eye movement (REM). The classifications of various sleep stages are made based on the physiological parameters and brain wave activity recorded using an electroencephalogram (EEG). More than one cycle of NREM and REM sleep stages may occur throughout the night of a healthy individual. The mentioned two sleep cycles alternate cyclically. In a sleep disorder, the irregularity

of cycling and/or devoid of any cycle stage may occur (Altevogt and Colten, 2006). (Figure 2.1)

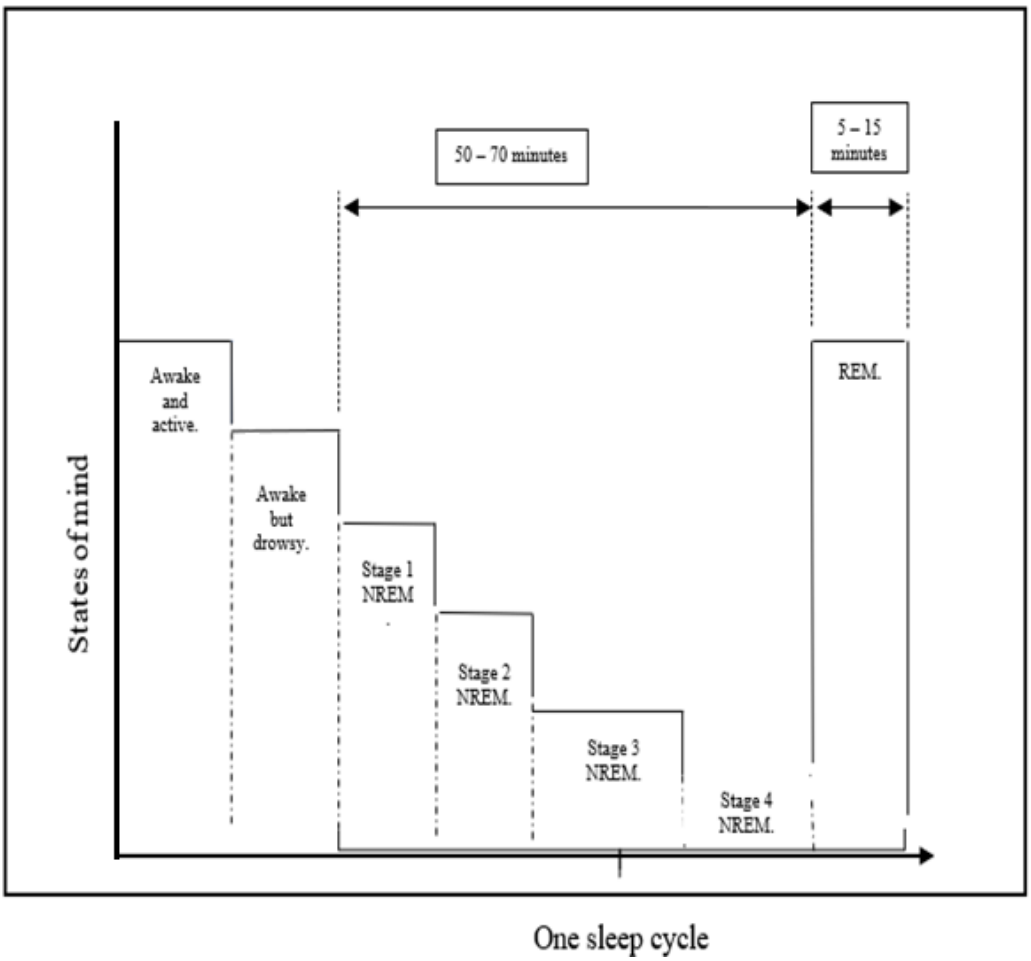


Figure 2.1: States of mind in awake and various sleep stages. There are four NREM stages, which move from light sleep to deeper sleep stage from one to four. After stage 4 is the REM sleep stage, where the states of mind resemble the awake and active stage. Adapted from Altevogt and Colten, 2006

A single sleep cycle consists of NREM, the initial stage, which is then followed by REM. The first cycle lasts for an average of 70 to 100 minutes, and the subsequent cycles are longer, with an average of 90 to 120 minutes per cycle. NREM sleep stage accounts for about 75 – 80% of the cycle, and 20 – 25 % is the REM sleep stage (Altevogt and Colten, 2006).

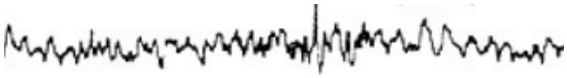
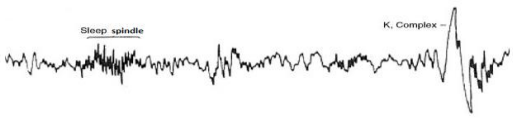
NREM sleep is further subdivided into stages 1, 2, 3, and 4. This subdivision is based on the EEG pattern observed (Table 2.1 – NREM sleep stages brain wave). From the active brain wave during wakefulness to the alpha wave, an active but clam or resting stage of the brain in Stage 1 of NREM. Stage 1, which lasts for about seven minutes, is easily interrupted by noise. The second stage of NREM has a lower voltage of brain wave and the main difference from Stage 1 is the presence of the K wave complex and sleep spindle (Table 2.1). Stage 3 and 4 are collectively called slow-wave sleep (SWS). They have high voltage and slow activity brain waves. Stage 4 has a higher voltage wave, and it lasts longer than Stage 3. Brain waves in NREM sleep are well synchronised (Altevogt and Colten, 2006).

REM sleep cycle started when the brain waves desynchronised, with low-voltage waves with mixed frequencies. The brain waves in a REM sleep cycle is described as having a sawtooth appearance. Along with the sawtooth brain waves activity, the muscles atonia and rapid eye movement occur in the REM sleep cycle. This stage lasts only for about one to five minutes during the initial cycle but getting longer in subsequent cycles. Unlike NREM sleep, REM sleep is not divided into any substages.

Dreaming is mostly associated with the REM sleep stage, during which the muscles atonia are important to avoid muscle actions during dreaming (Altevogt and Colten, 2006, Carskadon, 2011). Sleepwalking disorder is related to dreaming in the

latter two stages of NREM sleep, where the muscle tone is present (Guilleminault et al., 2006).

Table 2.1: Brainwaves in various states of mind and stages of sleep (Adapted from Altevogt and Colten, 2006).

State of mind	Brainwave	Features
Awake and active		Beta brain wave.
Awake and drowsy		Alpha brain wave.
Stage 1 of NREM		A mixture of alpha and theta waves.
Stage 2 of NREM		The appearance of sleep spindle and K-complex in theta wave.
Stage 3 of NREM		A mixture of theta and delta wave.
Stage 4 of NREM		Delta wave.
REM		Fast active brain wave. Resembles brainwave in active awake brain.

2.1.2 Physiological Benefits of Sleep

Although the actual functions of sleep are elusive, there are a few theories and ongoing studies that have been done regarding the functions of sleep for the human body. Researchers are still working on a few hypotheses about the actual functions of sleep. They mentioned the hypothesis about energy conservation in sleep. Energy conservation is related to the release of specific genes regulated based on the circadian cycle that controls the food or energy released during sleeping (Sen et al., 2017, Schmidt et al., 2017). Meanwhile, brain metabolic down-regulation is related to the NREM sleep cycle, which is postulated to preserve and restore the brain glucose level during sleep. However, brain metabolism is higher during the wake-up stage of the REM sleep cycle; this indicates more neuronal excitation occurs during the REM sleep stage (Krueger et al., 2016). This explains how a daytime power nap that involves only Stage 1 and 2 of NREM sleep stages has a rejuvenating body effect (Hayashi et al., 2005).

Sleep helps in body recuperation after being affected by certain diseases by enhancing the immune systems (Krueger et al., 2016). One of the most interesting functions of sleep is how it is related to many aspects of brain wellness. For instance, cognitive impairment is related to poor sleep quality and sleep insufficiency. A study showed that sleep insufficiency in adolescents is related to more significant risk-taking behaviour due to poor cognitive judgment (Telzer et al., 2013). Good quality of sleep is crucial for emotional stability, proved by the amygdala's negative stimulation in the limbic system of a sleep-deprived person (Yoo et al., 2007). And for learning and memory benefits, sleep-associated neuroplasticity is currently being one of the major neuroscience fields. Neuroscientists are working out at the various levels of how sleep affects learning and memory.

2.1.3 Sleep-Wake – Regulating System

Some essential brain areas are involved in sleep-wake regulation. Four regions are located in the hypothalamus, one in pineal gland, and one region is in the brainstem. In the hypothalamus, there are suprachiasmatic nucleus (SCN), lateral hypothalamus, ventral preoptic nucleus and tuberomammillary nucleus. SCN is the region involved in sleep-wake homeostasis and is related to the circadian rhythm. Sleep homeostasis determines the amount of sleep needed. It is explained when a person has been awake for a longer duration, that person will have a stronger sleeping drive. In turn, the sleeping drive decreases after a period of sleep (Krystal et al., 2013). In the lateral hypothalamus, orexin, also known as hypocretin, is the neuropeptide involved in wakefulness. They start firing during the sleep-wake transition. Narcolepsy, a chronic sleep disorder is characterised by extreme daytime sleepiness, is associated with orexin deficiency (Krystal et al., 2013, Kalia, 2006, Krebs, 2012).

The ventrolateral preoptic (VLPO) nucleus of the hypothalamus is important for the slow-wave sleep oscillation, the NREM stages. Neurons in the VLPO nucleus are activated by adenosine and prostaglandin D2. The tuberomammillary nucleus of the hypothalamus, mediated by the histamine neurotransmitter, is essential in promoting wakefulness (Kalia, 2006). Hence this is explained when drowsiness is the effect of anti-histamine medication.

The suprachiasmatic nucleus (SCN) of the hypothalamus acts as a regulator for the endogenous biological clock by controlling the wake-sleep cycle known as the circadian rhythm. SCN is located at the anterior hypothalamus. It serves as a ‘master clock’ that controls both physiological and behavioural circadian rhythm. SCN is a neural substrate for day and night cycles in many physiological regulations, including motor activity, body temperature secretion of many hormones, sleep-waking, and

many more (Gray, 2005, Krebs, 2012). SCN operated as a luminance detector when they received light input from the retina through retinothalamic fibres that terminate in SCN of the hypothalamus and regulate all physiological regulation based on it. The retinal ganglion cells' activation upon receiving light in the retina stimulates the melanopsin in the SCN. This triggers the sympathetic nervous system's activation in the thoracic region, leading to a negative feedback loop mechanism towards inhibition of melatonin released by the pineal gland (Kalia, 2006).

A region known as reticular formation has an ascending reticular activation system (ARAS) in the brainstem. This system has two pathways known to induce wakefulness. The deactivation of these pathways leading to sleep generating process. The pathways are the dorsal ascending pathway and the ventral ascending pathway. Fibres in the dorsal ascending pathway arise from the nuclei in the pons and medulla of the brainstem. They ascend towards the thalamic nuclei, and then they are projected to several parts of the brain cortex. Acetylcholine is the neuropeptide that mediates neuronal firing in this region during wakefulness and during the REM sleep stage. Fibres in the ventral ascending pathway arise from locus coeruleus, known as a noradrenergic neuron, while dorsal and median raphe nuclei are called serotoninergic neurons. They project toward the basal forebrain by passing by the lateral hypothalamus. Neurons of these fibres actively fire during wakefulness and not in REM sleep or REM sleep stages (Kalia, 2016). Lesions involving ARAS causes unconsciousness.

2.1.4 Neuromodulator for Wake-Sleep Cycle

Having known the regions involved in the sleep-wake regulating system, the neurotransmitter released by neurons in these regions is in charge of maintaining wakefulness or actively being released during sleeping. So, the neurons involved in

maintaining wakefulness are called the wake-active neurons, and neurons that are released during sleep are called sleep-active neurons (Szymusiak and McGinty, 1989, Morairty et al., 2004). Noradrenaline, histamine, and orexin are the neurotransmitters released by the neurons categorized in the wake-active neurons. Their release is abundant during awake, reduced during SWS, and ceased during REM sleep. Acetylcholine in the same group as neurotransmitters above, being actively released during the wake and REM sleep stage but decreases during SWS. The neurons in the sleep-active category release GABAergic neurotransmitter. They are mainly found in the basal forebrain and preoptic (VLPO) of the hypothalamus and reciprocal to the wake-active neurons (Jones, 2005).

2.1.5 Sleep Initiation Process

A passive closure of the cerebral sensory gate leads to deafferentation. This quiet initiation of sleep shuts up all the excitatory event of all wake-active neurons in the ARAS and all regions involved in arousal. The neurons in the preoptic or anterior hypothalamus that contain inhibitory neurotransmitters influence the wake-active neurons. The deactivation of ARAS stopping the sensory impulse firing towards the cerebral cortex (Datta, 2010).

Another theory mentioned the accumulation of endogenous metabolites that facilitates the movement from wakefulness to sleep. This theory is based on the homeostatic regulation of sleep, which means the longer the awake period, the higher the metabolites accumulate. The known metabolites that initiate the SWS are adenosine, prostaglandin D2, neuroinhibitory amino acid GABA, cytokine (interleukin I beta), and tumour necrosis factor α (TNFα) (Datta, 2010, Datta and Maclean, 2007).

2.2 Circadian rhythm

2.2.1 The molecular event under the circadian rhythm regulation

An endogenous circadian clock controls the circadian rhythm. The study of genetic factors that influence the circadian rhythm was done a few decades ago. In 1988, they finally found the first clue to how our body controls the biological clock through a fruit fly study when they found a protein named period (PER). This protein was scattered throughout the fruit fly cells' nucleus in a profound amount during the night time than in the day time, and it shuttles between cytoplasm and nucleus. The PER mRNA was also circulating high at night, indicating PER level is high in the presence of increased mRNA PER and PER oscillation follow the day and night cycle (Saez and Young, 1988).

In 1995, they discovered another gene, timeless (TIM). TIM and TIM mRNA circulate in a circadian manner like PER. TIM directly reacts in PER by preventing degradation of PER in the day time. In humans, the oscillator of circadian rhythm is regulated by a transcription-translation feedback loop (TTFL) (Sehgal et al., 1994, Price et al., 1995).

PER and TIM proteins transcription are activated by another gene call clock (CLK) and cycle (CYC) genes. These two genes interact with each other in the PER gene and produce mRNA for PER. On the other hand, PER and TIM is the negative regulator for CLK and CYC genes, thus promoting a circadian feedback loop cycle (Jin et al., 1999).

Besides the CYC and CLK that activate PER, the degradation of PER is done by a protein kinase known as doubletime (DBT). DBT phosphorylates PER and degrades them. Meanwhile, the TIM protein degradation occurs by the interaction of TIM with cryptochrome (CRY) protein. CRY genes that interact with the TIM in the

process of degradation of TIM are activated by light. That results in a gradual degradation of TIM as the morning arrives. On the other hand, leaving PER protein awaiting the time to be phosphorylated. The transcription and degradation of PER and TIM cycling followed the circadian rhythm and controlled the internal biological events of the organisms (Price et al., 1998, Jin et al., 1999).

2.2.2 Zeitgeber time

The exogenous factor that synchronises the organism's endogenous circadian rhythm is known as Zeitgeber. The factor can be a form of light, social and chemical. Zeitgeber time is a system that refers to the time in the period of Zeitgeber, for instance, the 12:12 light: dark cycle used in light factor Zeitgeber. The Zeitgeber time 0 (Zt 0) refers to the beginning of the light on, and ZT 12 is the beginning of the light off. (Daan and Merrow, 2002)

2.3 Memory

Memory is a part of learning where its quality is evaluated based on the performance of a person after a series of training. Memory involves the processes of data acquisition, consolidation, retention, retrieval, and performance at various stages and occurs at the cellular and molecular level of the human brain complex (Abel and Lattal, 2001). Memory acquisition happens during training, followed by up to a few days of memory consolidation and memory retrieval during re-training. Good memory consolidation is indicated by improvement in the last training session.

External sensory signals gain from learning or training will be received and transcribed in the brain cortex. The transcribed signal will then be transmitted to the hippocampus, where new memory formation occurs. After a strong or repeated signal transmission at the hippocampus, the recent memory is established. This memory is

then wired back to the brain cortex for storage. Disorder of hippocampus structure and function lead to the inability to produce recent memory while they still have the storage of the old one.

2.3.1 Memory classification

Memory is subjected to different ways of classification. One of the most common ways is dividing it into two main prongs: declarative and non-declarative memory (Figure 2.2). Neuropsychologists defined this classification based on the involvement of the medial temporal lobe's region during memory acquisition. Declarative memory critically involves encoding at the brain's medial temporal lobe region during the memory acquisition process (Rasch and Born, 2013). Hippocampus located at the medial temporal lobe is where the new memory formation occurs. Meanwhile, the entorhinal, perirhinal, and parahippocampus are the areas of memory storage. Thus, declarative memory is also known as hippocampal-dependent memory (Squire et al., 2004). Declarative memory is further divided into episodic and semantic memory. Episodic memory is the memory of events, for instance, the memory of our first day in university, and semantic memory is the general knowledge or fact that we learn in school, like the capital city of certain countries. Non-declarative memory is regarded as an unconscious type. It is more for procedural kinds like how to ride a bicycle (Squire, 1992).

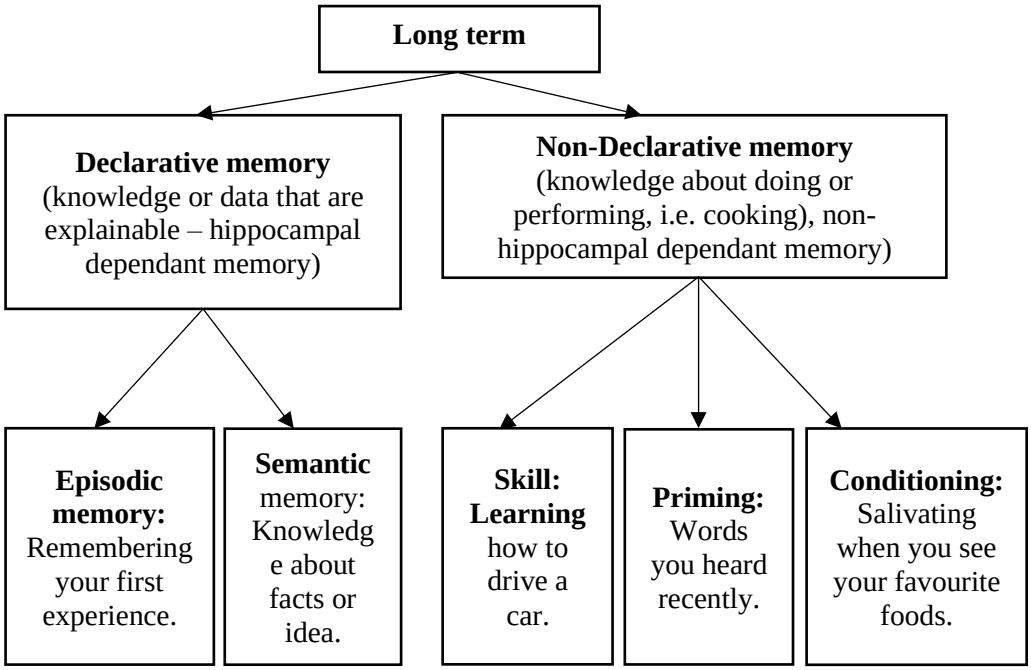


Figure 2.2: This figure depicted the classification of memory. Memory is classified as long and short-term memory. Long-term memory is further divided into declarative in the form of data or theory, while non-declarative is best explained as acquired skills.

2.3.2 Memory Stages

Memory processing involves three main stages, which are memory acquisition, memory consolidation, and memory retrieval. Memory acquisition is the stage where we gain new information or data from learning or training. Through a strong or effective way of the information or data collected, it will stay in our memory once it stabilised in storage, which is the brain cortex. The process of stabilisation is called the memory consolidation stage. Next is when we want to retrieve the information or data that we stored, we will recall the data from our memory. The latter is known as a memory retrieval stage.

2.3.3 Cellular and Molecular Level of Memory Formation

Neurons in the nervous system that connected at a small cleft are called a synapse. A typical neuron can have thousands of synapses connecting to other neurons. The connections work in an intricate manner that allows the brain to function normally. The synaptic connection can change over time. Following repeated stimulations, the connection can become more substantial, and it even may produce a new pair of synaptic connections. In the case of ignorance or without further stimulus, the synapse connection may weaken and eventually be lost. The ability of neurons to change the structure of their synaptic ends is known as neuronal plasticity. The established, recent memory is formed after a robust synaptic connection. The strength of the connection is measured by the level of excitability of the postsynaptic neurons. A strong through repeated and high-frequency stimulus strengthens the synaptic connection after some time. This is known as long term potentiation (LTP), a cellular basis of learning and memory. (Abel and Lattal, 2001)

LTP can occur at various locations in the brain. One of them being the hippocampus, where LTP in this site involves glutamate as their neurotransmitter.

Glutamate is the most common excitatory neurotransmitter in the central nervous system. Neurons with glutamate neurotransmitters are known as glutamatergic neurons. Stimulations of glutamatergic neurons lead to the action's potential travels down through its axon and glutamate releases at its synaptic end. Glutamates bind to the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor and N-methyl-D-aspartate (NMDA) receptor at the postsynaptic neurons (Abel and Lattal, 2001).

When a weak signal is transmitted in the presynaptic neuron, it releases a small number of glutamates. Those glutamates will bind to both AMPA and NMDA receptors and allow AMPA receptors to influx cations, sodium (Na^+), and no cations influx through NMDA receptors due to magnesium (Mg^{2+}) blockage in the NMDA receptor channel. These amounts of Na^+ produce weak depolarisation of the postsynaptic neuron. However, a strong signal will release many glutamates and allow more Na^+ influx through the AMPA receptor. An adequate amount of Na^+ allowed greater depolarisation of postsynaptic neurons, which removes the Mg^{2+} blockage of the NMDA receptor. When the NMDA channel opened, it will enable the influx of Na^+ and calcium (Ca^{2+}). Calcium is the mediator of LTP induction by initiating the signaling pathway that activating several protein kinases (Figure 2.3). Calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PCK) are two types of protein kinases in early LTP. In early LTP, those protein kinases phosphorylate the AMPA receptor increases the AMPA receptor conductions to sodium. Protein kinases help in bringing out more AMPA receptors from the intracellular storage to the postsynaptic membrane. This signal of early LTP is the basis of short-term memory, which lasts for several hours. In the late LTP phase, it involves new protein synthesis, which enhances the connection between pre and

postsynaptic neurons. It includes the synthesis of new AMPA receptors and proteins involved in the formation of new synaptic dendrites and synaptic connections. The late phase of LTP is the basis of long-term memory formation (Abel and Lattal, 2001).

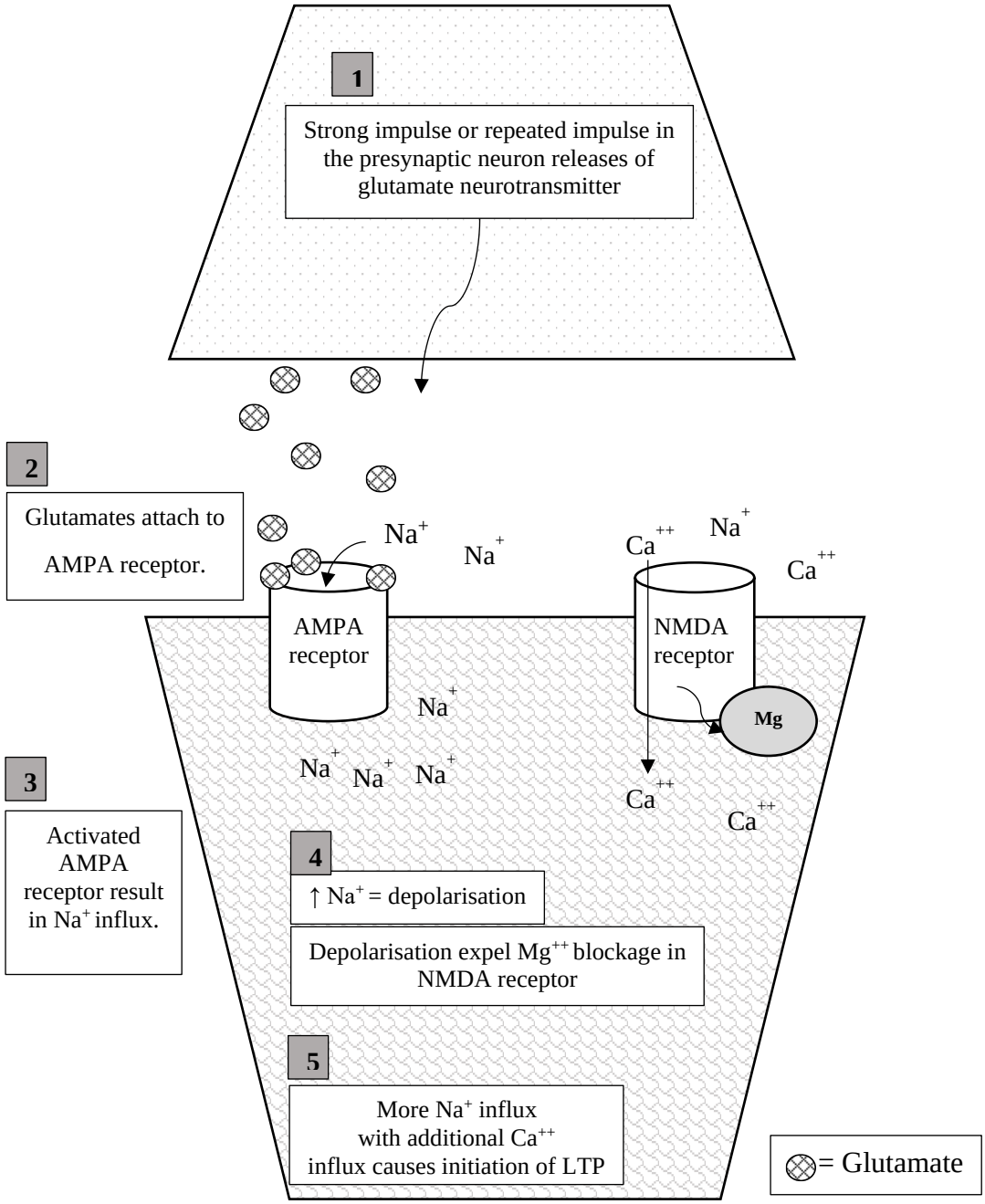


Figure 2.3: Diagram of the molecular mechanism of Long Term Potentiation (LTP) initiation in neuronal plasticity. It is started with the strong or repeated impulse from the presynaptic neuron that releases the glutamate neurotransmitter. Those glutamates are then attached to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor, which results in the influx of sodium ions through the receptor. The increased concentration of sodium ion causes depolarisation and, at the same time, magnesium blockage at N-methyl-D-aspartate (NMDA) receptor was removed resulting in the influx of calcium ion and eventually initiate the LTP (Adapted from Abel and Lattal, 2001).

2.4 Sleep and Memory

Memory acquisition is an initial step in-memory processing. Acquisition of memory occurs during learning or training, and it relies on the integrity of the prefrontal cortex functions (PFC). Memory encoding is a process that occurs during the wake-up state. The deprivations of the pre-training sleep affecting the PFC metabolic rate, thus causing the memory acquisition difficulty (Walker and Stickgold, 2006). The cerebral metabolic rate is measured based on the oxygen level in the cerebral blood flow (Clarke, 1999). During SWS, the rate recorded slightly decreased than in the state of wakefulness but increased during the REM sleep stage (Madsen and Vorstrup, 1991, DiNuzzo and Nedergaard, 2017). While other researchers support that consolidation of memory is supported by sleep through various experiments (Ahrberg et al., 2012, Genzel et al., 2014). Post-training sleep stabilises newly obtained memory by increasing its resistance to interference (Ellenbogen et al., 2006). This allows this memory to stay longer before consolidation takes place. Sleeping after memory acquisition has better memory retrieval than wakefulness (Korman et al., 2007).

2.4.1 Roles of Sleep on Memory Consolidation

For more than a hundred years, scientists have established the importance of sleep in memory consolidation through thousands of research. Among those, the most acceptable finding is the ability to sleep to stabilize memory by increasing memory interference from external stimuli. From the results of REM sleep's involvement, they revealed the importance of SWS for the consolidation of memory. Memory acquisition allows the memory encoding process in the brain. During the SWS stage, optimisation of memory encoding occurs, and consolidation occurs during the REM sleep stage (Rasch and Born, 2013).

It is described that memory storage in declarative memory involved two standard storages: fast learning and slow learning storage. Fast learning store refers to the hippocampal that is engaged in new memory formation. A slow learning store is long-term memory storage located at the neocortex. Fast learning store receives new data and stores it temporarily in it. In other recent data, it encoded the previous data that may sometimes be unstable and easily disrupted by the new ones. So, because of sleep adequacy, pre-learning sleep is found to strengthen the fast learning storage center by making it more stable and resistant to deletion upon other data encoding.

On the other hand, going to sleep after learning makes the memory storage more stable before its projection to the slow learning storage after a certain learning period (Rasch and Born, 2013). To prove the importance of REM sleep in stabilising the newly stored memory, Davis et al., (2006) have studied REM sleep deprivation's effects on the up-regulation of cortactin, an important cytoskeleton in neuronal plasticity. Their study confirmed the incidence of inhibition of up-regulation of the cortactin, which leads to interference of LTP in rats and the whole process in memory consolidation (Davis et al., 2006).

2.4.2 Molecular Modulator for LTP in Memory Consolidation

A few molecules and proteins are critically crucial for the modulation of LTP in hippocampal-dependent memory consolidation processes. They are the brain-derived neurotrophic factor (BDNF), cAMP response-element-binding (CREB), and calcium–calmodulin-dependent protein kinase II (CAMKII). These molecules involve in many directions of strengthening synaptic plasticity.

BDNF is a growth factor for neurons in the nervous system. Thus, it is known as a neurotrophic factor. BDNF, translated from the chromosome in the human brain, binds to tyrosine kinase B (TrkB) and low-affinity nerve growth factor receptor

(LANGF) before eliciting their action, neuronal growth, and maturity. BDNF is crucial for the growth and survival of dopaminergic, GABAergic, serotoninergic, and cholinergic neurons, which are important for neuronal excitatory (Pillai, 2008). In the LTP signaling process, BDNF in the presynaptic neurons enhances the release of neurotransmitters. Meanwhile, in postsynaptic neurons, they increase the influx of calcium and potassium ion through enhancing the ion channels in NMDA receptors and eventually helps in the initiation of LTP through the early and late phase of LTP (Poo, 2001, Lu et al., 2008) cited by (Autry and Monteggia, 2012).

CREB is a cellular transcription factor that binds to DNA and influences the up-regulation or down-regulation of certain genes. BDNF gene is one of the genes that CREB regulates. A previous study that involved the disruption of the CREB gene leads to impairment of long-term memory formation (Bourtchuladze et al., 1994). CREB involve in molecular cascades mediating the transformation of short-term memory to long-term memory. They influence synaptic plasticity during long term memory consolidation. Besides, it is also found that they increase neuronal excitability (Benito and Barco, 2010). As mentioned in LTP cellular mechanism, CREB is a factor that is opening the gate for protein phosphatases that is important in maintaining the influx of sodium through AMPA receptor for induction of powerful depolarisation, cascading for the LTP initiation (Blitzer et al., 1995).

CAMKII is a protein kinase that regulates the calcium/calmodulin complex. It is involved in many cellular signaling cascades, and one of them is in learning and memory. Regulation of this protein kinase induces protein phosphorylation is a catalyst for various intracellular LTP induction changes in a neuron. The intracellular function includes neurotransmitter synthesis, cytoskeletal functions, gene expression, synaptic plasticity and all process involved in LTP (Yamauchi, 2005).

2.5 Circadian Rhythm and Memory

The circadian rhythm is controlled by a central pacemaker, the SCN of the anterior hypothalamus, is associated with the hippocampal formation. Hippocampus is critical in learning and memory, integrating the circadian information through direct or indirect input from SCN or other oscillators input, modulating the hippocampal neurogenesis through clock genes expression and genetic modification. (Smarr et al., 2014, Ruby et al., 2008). A study concluded that the memory impairment in circadian rhythm disruption is associated with inhibition of GABAergic communication to the hippocampus (Ruby et al., 2008). Clock gene expression in the hippocampus, which is rhythmically oscillated postulated to be synchronised by SCN (Smarr et al., 2014). These clock genes then regulate the hippocampus's neurogenesis (Gery et al., 2006, Matsuo et al., 2003). Melatonin is an endogenous hormone released based on the circadian clock, where its levels are higher during the dark phase, promoting neuron maturation and survival (Ganguly et al., 2002).

2.6 Hippocampus and Memory

The cerebral cortex is the largest part of the brain, which contains lobes that include the limbic lobe. The limbic lobe, located at deep brain area is also known as the medial temporal lobe in the cerebral hemisphere's medial wall. The limbic lobe is made of subcallosal (corpus callosum), cingulate and para-hippocampal gyri (Figure 2.4).