THE ROLE OF TUALANG HONEY IN MODULATING NOCICEPTIVE RESPONSES IN THE THALAMUS OF RAPID EYE MOVEMENT (REM) SLEEP DEPRIVATION RAT MODEL

ANIS SYAHIRAH BINTI MOHD SHAFIE

UNIVERSITI SAINS MALAYSIA

2022

THE ROLE OF TUALANG HONEY IN MODULATING NOCICEPTIVE RESPONSES IN THE THALAMUS OF RAPID EYE MOVEMENT (REM) SLEEP DEPRIVATION RAT MODEL

by

ANIS SYAHIRAH BINTI MOHD SHAFIE

Thesis submitted in fulfilment of the requirements

for the degree of

Master of Science

JANUARY 2022

ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisors, Assoc. Prof. Dr. Che Badariah Abd Aziz, Dr Liza Bt Noordin and Dr Idris Long for continuous support until the completion of my research project, for their patience, motivation, enthusiasm and immense knowledge. Their guidance has assisted me throughout my study period until the completion of the thesis. I would also like to express my gratitude for their encouragement, insightful comments and effective suggestion to improve my research project and thesis writing. Apart from that, I would like to thank the Ministry of Higher Education Malaysia for providing the Fundamental Research Grant Scheme (FRGS) grant [FRGS/1/2018/SKK08/USM/02/15] to support the MSc. research project.

Plus, my sincere gratitude to support staff in Physiology, Animal Research and Service Centre (ARASC) and Central Research Laboratory of School of Medical Science, Universiti Sains Malaysia, for their assistance and allowing me to use the equipment in the lab during my study period. Finally, I would like to express my gratitude to my parents, Mohd Shafie Bin Ismail and W Kalifatra Binti Wan Ali and my siblings. Lastly, I would like to thank my lab mates for their wise counsel and sympathetic ear. They also provide me unfailing support and continuous encouragement throughout my study. This accomplishment would not be possible without them. Thank you.

TABLE OF CONTENTS

CHAPTER 2- LITERATURE REVIEW

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$^1O_2$	Singlet oxygen
5HT	5-hydroxytryptamine
ANOVA	Analysis of variance
ARASC	Animal Research and Service Centre
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BHT	Butylated hydroxytoluene
BWg	Body weight gain
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
Cd	Cadmium
CI	Confidence interval
COX-2	Cyclooxygenase 2 or Prostaglandin-endoperoxide synthase 2
CPG-15	Candidate plasticity-related gene 15
CVD	Cardiovascular diseases

dH_2O	Distilled water
DNA	Deoxyribose nucleus acid
DNTB	Dinitrothiocyanobenzene
DPGi	Dorsal paragigantocellular reticular nucleus
DREAM	Downstream regulatory element antagonistic modulator
ECG	Electrocardiography
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
EOG	Electrooculography
Fc	Food consumption
FAMA	Federal Agricultural Marketing Authority
FMC	Free moving control
GABA	Gamma aminobutyric acid
GDH	Glutamate dehydrogenase
GiA	Alpha gigantocellular reticular nucleus.
GiV	Ventral gigantocellular reticular nucleus

GLUT1	Glucose transporter 1
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
H_2O	Water
H_2O_2	Hydrogen peroxide
HRP	Horseradish peroxidase
H&E	Hematoxylin and eosin
IASP	International Association for the Study of Pain
IFN-y	Interferon gamma
IL-1	Interleukin-1
IL-6	Interleukin-6
LPGi	Lateral paragigantocellular nucleus
LTD	Long-term depression
LTP	Long-term potentiation
MDA	Malondialdehyde

MOA	Monoamino oxidase
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSP	Multiple small platforms
NA	Noradrenergic neurons
NF-κB	Nuclear factor kappa B
NK1	Neurokinin 1
NMDA	N-methyl-D-aspartate
NMDA R	N-methyl-D-Aspartate receptor
NR1	NMDA receptor 1
NR2	NMDA receptor 2
NR3	NMDA receptor 3
$NO^{\bullet}$	Nitric oxide
NOS	Nitric oxide signalling
NR2B	NMDA receptor 2B
O_2	Oxygen
$O_2^{\bullet -}$	Superoxide anion radical
OD	Optical density

$OH^{\bullet}$	Hydroxyl radical
PAG	Periaqueductal gray
PCR	Polymerase chain reaction
PBS	Phosphate buffered saline
PPT/LDT	Pedunculopontine and laterodorsal tegmental
PSG	Polysomnography
REM	Rapid eye movement
REMsd	REM sleep deprivation for 72 hours
REMsdH	REM sleep deprivation for 72 hours pretreated with Tualang honey
R^2	Correlation coefficient
RN	Raphe nuclei
ROS	Reactive oxygen species
RT	Reticular thalamic nucleus
RVM	Rostroventromedial medulla
SEM	Standard error of mean
S1	Primary somatosensory
SLD	Sublaterodorsal

SOD	Superoxide dismutase
SPSS	Statistical Package for Social Sciences
STZ	Streptozotocin
TBA	Thiobarbituric acid
TC	Tank control
TNF-α	Tumour necrosis factor alpha
TRN	Thalamic reticular nucleus
UCP1	Uncoupling protein 1
VB	Ventrobasal nucleus
VLPO	Ventrolateral preoptic
VPL	Ventral posterolateral tract
ZI	Zona incerta

PERANAN MADU TUALANG TERHADAP MODULASI TINDAK BALAS NOSISEPTIF DALAM TALAMUS MODEL TIKUS KEKURANGAN TIDUR REM

ABSTRAK

Kekurangan tidur REM telah dirumuskan boleh menyebabkan perubahan kepada tindak balas nosiseptif; walaubagaimanapun sebab dan kesannya masih kurang difahami. Kajian ini bertujuan untuk menyelidik kesan kekurangan tidur REM ke atas skor tingkah laku nosisiseptif, perubahan histologi, paras parameter tekanan oksidatif dan paras reseptor NMDA R2 di dalam talamus model tikus kekurangan tidur REM. Seterusnya, kajian ini juga mengenalpasti kesan madu Tualang terhadap parameter yang dikaji. Empat puluh lapan ekor tikus jantan Sprague Dawley telah dibahagikan secara sama rata kepada empat kumpulan (n=12); kawalan bebas-gerak (FMC), kekurangan tidur REM selama 72 jam (REMsd), kekurangan tidur REM selama 72 jam yang dirawat dengan madu Tualang (REMsdH) dan kawalan tangki (TC). Madu Tualang (1.2 g/ kg berat badan/hari) telah diberi secara oral selama tiga puluh hari sebelum prosedur kekurangan tidur REM dijalankan. Selepas tempoh eksperimen, ujian formalin dan ujian tingkah laku nosiseptif telah dilakukan. Tikus telah dikorbankan, dan talamus telah dikeluarkan untuk pemeriksaan histologi dan pengukuran paras parameter tekanan oksidatif dan reseptor NMDA R2. Kumpulan REMsdH menunjukkan penurunan yang ketara dalam ujian tingkah laku nosiseptif berbanding kumpulan REMsd (p<0.05). Bilangan neuron Nissl positif menunjukkan peningkatan yang ketara dalam REMsdH berbanding REMsd (p<0.05). Neuron gelap dapat dilihat dalam kawasan VPL pada kumpulan REMsd, tetapi tiada dalam kumpulan REMsdH. REMsdH menunjukkan peningkatan ketara (p<0.001)

dalam paras GSH, GR, SOD dan CAT tetapi penurunan ketara (p<0.001) dalam paras MDA dan reseptor NMDA R2 dalam talamus REMsdH berbanding REMsd. Kesimpulannya. pemberian madu Tualang dapat memberi kesan perlindungan terhadap kesan buruk akibat kekurangan tidur REM.

THE ROLE OF TUALANG HONEY IN MODULATING NOCICEPTIVE RESPONSES IN THE THALAMUS OF RAPID EYE MOVEMENT (REM) SLEEP DEPRIVATION RAT MODEL

ABSTRACT

Rapid eye movement (REM) sleep deprivation has been postulated to contribute to the alteration of nociceptive responses; however, the causes and effects are poorly understood. The aim of this study was to investigate the effects of REM sleep deprivation on nociceptive behaviour score, histological changes, the level of oxidative stress parameters, and the level of NMDA R2 receptors in the thalamus of REM sleep-deprived rat model. Furthermore, this study also determined the effects of Tualang honey on the parameters investigated. Forty-eight Sprague-Dawley male rats were equally divided into four groups (n=12); free moving control (FMC), REM sleep deprivation for 72 hours (REMsd), REM sleep deprivation for 72 hours pretreated with Tualang honey (REMsdH) and tank control (TC). Tualang honey (1.2 g/kg body weight/ day) was given by oral gavage for 30 days prior to the REM sleep deprivation procedure. Following the experimental period, formalin test and nociceptive behaviour were conducted. The rats were sacrificed, and the thalamus was removed for histological examination and quantification of oxidative stress parameters and NMDA receptors levels. REMsdH group showed a significant decrease in nociceptive behaviour score compared to REMsd group (p<0.05). The number of Nissl-stained neurons was significantly higher in REMsdH compared to REMsd (p<0.05). Dark neurons were observed in the VPL region of the REMsd group but not in the REMsdH group. REMsdH showed a significant increase (p<0.001) in GSH, GR, SOD, and CAT levels

but a significant decrease (p<0.001) in MDA and NMDA R2 levels in the thalamus compared to REMsd. In conclusion, the administration of Tualang honey has protective effects against the adverse effects of REM sleep deprivation.

CHAPTER 1

INTRODUCTION

1.1 Background

Sleep has a vital role in life and is important for homeostasis (Mir et al., 2019). There are two stages of sleep, rapid eye movement (REM) sleep and non-REM sleep (Huang et al., 2020). Sleep deprivation has become public health challenges (Chattu et al., 2019). Studies have shown the association of sleep deprivation with atherosclerosis (Cherubini et al., 2021) and multiple cardiovascular diseases (CVD) risk factors, including diabetes (Khandelwal et al., 2017) and hypertension (Li et al., 2020b). Sleep deprivation may lead to difficulties in concentration, visual disturbances, slower reaction time, poor memory (Silva et al., 2004), and may alter pain response. Studies on humans have shown the association of sleep deprivation with increased pain perception (Schrimpf et al., 2015; Iacovides et al., 2017) and pain responses such as neuropathic pain and fibromyalgia syndrome (Zelman et al., 2006; Mork and Nilsen., 2012).

REM sleep deprivation in human subjects has been reported to cause changes in the electroencephalogram (EEG) power spectra (Liu et al., 2021) and increased pain responses (Azevedo et al., 2011). In animal studies, REM sleep deprivation has been linked to increased c-fos (Terao et al., 2003), hyperalgesia (Ajao, 2011) and nuclear Downstream Regulatory Element Antagonistic Modulator (DREAM) protein expression in the thalamus (Siran et al., 2014). Various neurochemical changes have been reported to contribute to the modulation of pain responses, including alteration of serotonergic and GABAergic neurons, which are involved in the modulation of descending pain

pathway (Tao et al., 2019; Wei et al., 2020). Stressful conditions, including sleep deprivation, can cause an uncontrolled release of reactive oxygen species (ROS). Increased ROS can lead to cell dysfunction, tissue degeneration and modification of body physiological function. REM sleep deprivation has been linked to alteration of body physiology that is mainly caused by a high level of ROS (Pandey and Kar, 2018). However, the exact mechanism is still unknown. REM sleep deprivation have been shown previously to cause oxidative stress in the hippocampus (Ramanathan et al., 2002), hypothalamus, midbrain and hindbrain of rats (Mathangi et al., 2012).

The release of glutamate is modulated by the continuous firing of peripheral nociceptors that unblocks magnesium ions and activates the N-methyl-D-aspartate (NMDA) with a massive influx of calcium ions (Miller et al., 2011). The imbalance concentration of calcium ions disrupted the homeostasis of intracellular calcium ions (Maneshi et al., 2017). The hyperalgesia in inflammatory animal pain model was also modulated by N-methyl-D-aspartate (NMDA) receptors in the thalamus (Kolhekar et al., 1997; Hasim et al., 2020). The association between oxidative stress and the activation of NMDA receptors has been reported earlier (Brittain et al., 2012; Reyes et al., 2012). Thalamus is an important structure in the pain pathway that involves in the modulation of acute and chronic pain (Abd Aziz et al., 2005; Abd Aziz and Ahmad, 2006). Thalamus is involved in the transmission of nociceptive information from the peripheral nervous system to the central nervous system for characterisation and localisation of nociceptive stimuli. In this study, ventral posterolateral (VPL) region of the thalamus was studied as the neurons of this region involved in synapsed activity (third-order neurons) of spinothalamic tract in ascending pain transmission (Al-Chalabi et al., 2020). Reports

have shown that REM sleep deprivation caused changes in thalamocortical functional connectivity in animal study (Shao et al., 2014), reduced thalamic grey volume in animal study (Somanath et al., 2021) and reduced thalamic grey volume in healthy volunteers (Liu et al., 2013). However, only a few studies focus on the effect of oxidative stress in the thalamus due to REM sleep deprivation.

Tualang honey administration in rats has been extensively studied as it may provide protective effects in oxidative stress (Erejuwa et al., 2010), inflammatory pain (Bashkran et al., 2011) and anticancer (Khalid et al., 2018). In comparison to other types of honey, Tualang honey is more effective as it contains higher flavonoids and phenolic content (Kishore et al., 2011; Khalil et al., 2012). Tualang honey's anti-inflammatory and antioxidant properties would benefit changes caused by REM sleep deprivation in rat models. Al-Rahbi et al. (2014) stated that Tualang honey modulates anxiety-like behaviour by its antioxidant property. Other than that, Tualang honey also minimises oxidative stress in the brain (Erejuwa et al., 2010) and protects brain morphology (Othman et al., 2015). Previous work by Hasim et al. (2020) revealed that administration of Tualang honey reduced pain behaviour score, improved morphology of neurons, reduced oxidative stress and levels of NMDA in the thalamus in offspring prenatally stressed rats. However, it remains unknown whether Tualang honey administration could provide a protective effect against the alteration of the thalamus due to REM sleep deprivation.

1.2 Problem statement

While there have been several studies on the effect of REM sleep deprivation on pain responses in various brain regions, the exact effects of REM sleep deprivation in the thalamus have yet to be published. It is not known whether REM sleep deprivation can cause changes in the pain score, oxidative stress level and NMDA level in the thalamus and whether Tualang honey administration during REM sleep deprivation has any protective effects on the nociceptive behaviour score, histology of thalamus, level of oxidative stress and level of NMDA receptors in the thalamus of REM sleep deprivation rat model.

1.3 General objectives

To evaluate the effects of REM sleep deprivation on nociceptive behaviour score, histological changes, oxidative stress parameters, and NMDA receptor level in the thalamus, and whether administration of Tualang honey would give protective effects in the rat model.

1.4 Specific Objectives

1. To determine the effect of REM sleep deprivation on nociceptive behaviour score in adult rats.
2. To determine the effect of REM sleep deprivation on histological changes of the thalamus in adult rats.
3. To determine the effect of REM sleep deprivation on the level of oxidative stress parameters in the thalamus of adult rats.

4. To determine the effect of REM sleep deprivation on the level of NMDA R2 in the thalamus of adult rats.
5. To determine the effects of Tualang honey on the parameters investigated in REM sleep deprivation rat model.

1.5 Hypothesis of the study

It is hypothesised that Tualang honey administration could reduce the nociceptive behaviour score, improved histology of the thalamus, reduced the levels of oxidative stress and NMDA R2 in the thalamus of the REM sleep-deprived rat model.

1.6 Justification of the study

REM sleep deprivation has been linked to oxidative stress and the activation of NMDA receptors in the central nervous system; however, the exact mechanisms of REM sleep deprivation leading to alteration of nociceptive response in rats are poorly understood. Whether REM sleep deprivation may affect the nociceptive behaviour score, the histology, the level of oxidative stress and the level of NMDA receptors in the thalamus need to be investigated. In addition, it is unknown whether there are protective effects of Tualang honey administration on the nociceptive behaviour score, histology, level of oxidative stress and NMDA receptors in the thalamus of REM sleep deprivation rat model.

CHAPTER 2

LITERATURE REVIEW

2.1 Sleep

Sleep duration and quality are important health determinants. Sleep plays a crucial role in regulating emotion, learning and memory (Perry et al., 2013). Reduced responsiveness to external stimuli, minimal body movement, reduced breathing rates, and stereotypic posture are characteristics of sleep (Roehrs and Roth, 2019). Polysomnography (PSG), electrooculography (EOG), electrocardiography (ECG), electroencephalography (EEG), and electromyography (EMG) are instruments that can be used to determine the alteration of body position and brain wave architecture (Carley and Farabi, 2016). Sleep initiation and maintenance required the suppression of ascending arousal system activity by inhibitory neurons in ventrolateral preoptic (VLPO), which remains active throughout sleep. In addition, the suprachiasmatic nucleus sent signals to VLPO regions, which play an essential role in the central circadian clock.

2.1.1 Stages of sleep

Sleep is subdivided into rapid eye movement (REM) sleep and non-REM sleep (NREM) (Huang et al., 2020). Non-REM sleep consists of three stages; Stage 1 (N1), Stage 2 (N2) and Stage 3 (N3). N1 is light sleep with theta waves ranging from 0 to 7 Hz. Meanwhile, stage N2 is marked with the presence of spindles waves and K-complexes. Stage N3 is also known as slow-wave sleep with theta waves ranging from 0.5 to 3 Hz. The skeletal muscle tone is the lowest during REM sleep stage, and it has a sharp theta

waves or wake-like EEG patterns. The cyclical sleep process begins with non-REM sleep and REM sleep occurring every 60 to 90 minutes throughout the night, as shown in Figure 2.1 (Scamell et al., 2017).

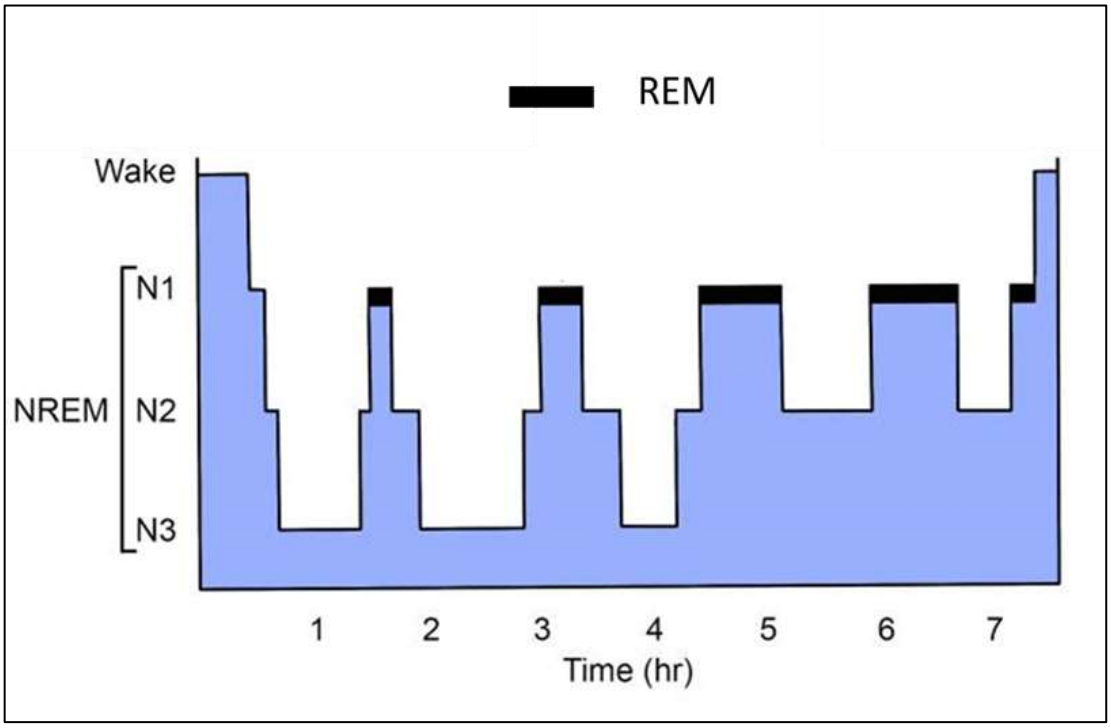


Figure 2.1 A hypnogram of the sleep cycle in a healthy young adult throughout the night. The sleep phases were cyclically alternated every 90 minutes from non-REM sleep to REM sleep. Modified from Scamell et al. (2017).

2.1.2 Physiological changes during REM sleep

REM sleep phase was first identified by Aserinsky and Kleitman in 1953 (Shepard et al., 2005). REM sleep is characterised by decreased higher voltage activity during the non-REM sleep period, measured using EEG. The EEG of REM sleep is similar to that of the waking state as the subject experienced a vivid dream during REM sleep. REM sleep is also known as paradoxical sleep because animals remained asleep and unconscious during this phase, but their EEG activities resembled a waking state. The muscles also become atonia during REM sleep, as evidenced by EMG. Other changes during REM sleep include irregular heart and respiratory rates, which do not occur during non-REM sleep (Siegel, 2005).

A sufficient amount of REM sleep is important for brain function, memory and learning. Peever and Fuller (2017) reported that during REM sleep, the twitches of muscle helped activate brain regions such as the hippocampus, cerebral cortex and red nucleus that is important for sensorimotor development. Previous studies have demonstrated the key roles of REM sleep in learning and memory as REM sleep is involved in the maintenance of new synapses involved in motor learning and the strengthening of newly formed postsynaptic dendritic spines (Peever and Fuller, 2017). REM sleep is essential for normal physiological function, such as for body temperature homeostasis. REM sleep is also important in normal physiological functions regulated by neurons in the brainstem, such as the essential role in emotion and protective effects toward stress situations in animal models. Besides memory consolidation, REM sleep is required for internal stimulation of adaptive behaviour after stressful situations (Suchecki et al., 2012).

2.1.3 REM sleep deprivation methods

Numerous methods to induce REM sleep deprivation in animals have been reported, including the classical single platform, double platforms, multiple small platforms (MSP), gentle handling, electrical stimulation and disk-over-water (Mohmed Nor et al., 2021). Single classical, double platforms and MSP used platforms in a glass tank, often an inverted flowerpot. The water is added and the level of the platform is one cm above a pool of water. Single classical, double platforms and MSP used one, double and multiple platforms (3 to 5 cm in diameter) respectively, placed at the center of the glass tank (Villafuerte et al., 2015). Animals lose their muscle tone during REM sleep and cannot keep their bodies on the platform, falling into the water and waking up immediately. Water and food were available *ad libitum* (Villafuerte et al., 2015). Many studies have utilised platform methods to evaluate the effects of REM sleep deprivation on the oxidant-antioxidant status in the hypothalamus (D'Almeida et al., 2000), behaviour (Hanlon et al., 2005), lipid peroxidation (Thamaraiselvi et al., 2012), endothelium (Nawi et al., 2020) and memory (Jung and Noh, 2021).

2.1.4 Regulation of REM sleep

The thalamus plays a key role in sleep and circadian rhythm. During REM sleep, functional neuroimaging proved that the thalamus nuclei remained active and relayed sensory information to the cerebral cortex (Jan et al., 2009). Other than that, the interaction between the reticular nucleus of the thalamus and thalamocortical neurons is involved in the production of rhythmic activity of sleep spindles during NREM sleep. During the early discovery of REM sleep, researchers believed that a neural circuit in the

pons was responsible for the regulation of REM sleep. However, discoveries had speculated that glutamatergic neurons in the sublaterodorsal nucleus (SLD) of the pons play a primary role in generating REM sleep.

Recent studies have also defined three pedunculopontine and laterodorsal tegmental nuclei (PPT/LDT) neurons population, which are cholinergic neurons, glutamatergic neurons and GABAergic neurons that are responsible for the transition of non-REM to REM and the regulation of REM sleep (Scammell et al., 2017). The transition from non-REM sleep to REM sleep started with the firing of cholinergic neurons. Cholinergic neurons such as acetylcholine were abundant in the dorsal pons during REM sleep similar to waking state. Meanwhile, glutamatergic neurons in the SLD are responsible for generating muscle atonia during REM sleep. The release of glutamatergic neurons stimulates GABAergic/glycinergic neurons in the ventromedial medulla and spinal cord that hyperpolarise motor neurons and cause muscle paralysis (Scammell et al., 2017). The REM sleep promoting and suppressing pathways are shown in Figure 2.2.

2.1.5 Relationship between sleep deprivation and pain

Sleep deprivation can be due to decreased quantity or quality of sleep (Medic et al., 2017). Decreased quantity occurs when the total time of sleep is reduced. Meanwhile, decreased quality of sleep occurs when there is arousal in the sleep fragmentation. Sleep deprivation is an important issue because it leads to decreased alertness and performance, and health problems (Cirelli and Tononi, 2019). Sleep deprivation was reported to increase pain perception (Schrimpf et al., 2015; Iacovides et al., 2017). Furthermore, sleep deprivation is associated with increased pain responses in patients

with chronic pain such as neuropathic pain and fibromyalgia syndrome (Zelman et al., 2006; Mork et al., 2017). Sleep deprivation has been linked to increased nociceptive responses in animals (Onen et al., 2001) and human studies (Lautenbacher et al., 2006; Roehrs and Roth, 2006). **A**nimal studies have reported that REM sleep deprivation was associated with increased c-fos (Terao et al., 2003), hyperalgesia (Araujo et al., 2011) and increased nuclear DREAM protein expression in the thalamus (Siran et al., 2014). However, very few studies have demonstrated changes in the thalamus that may contribute to the modulation of pain responses following sleep deprivation.

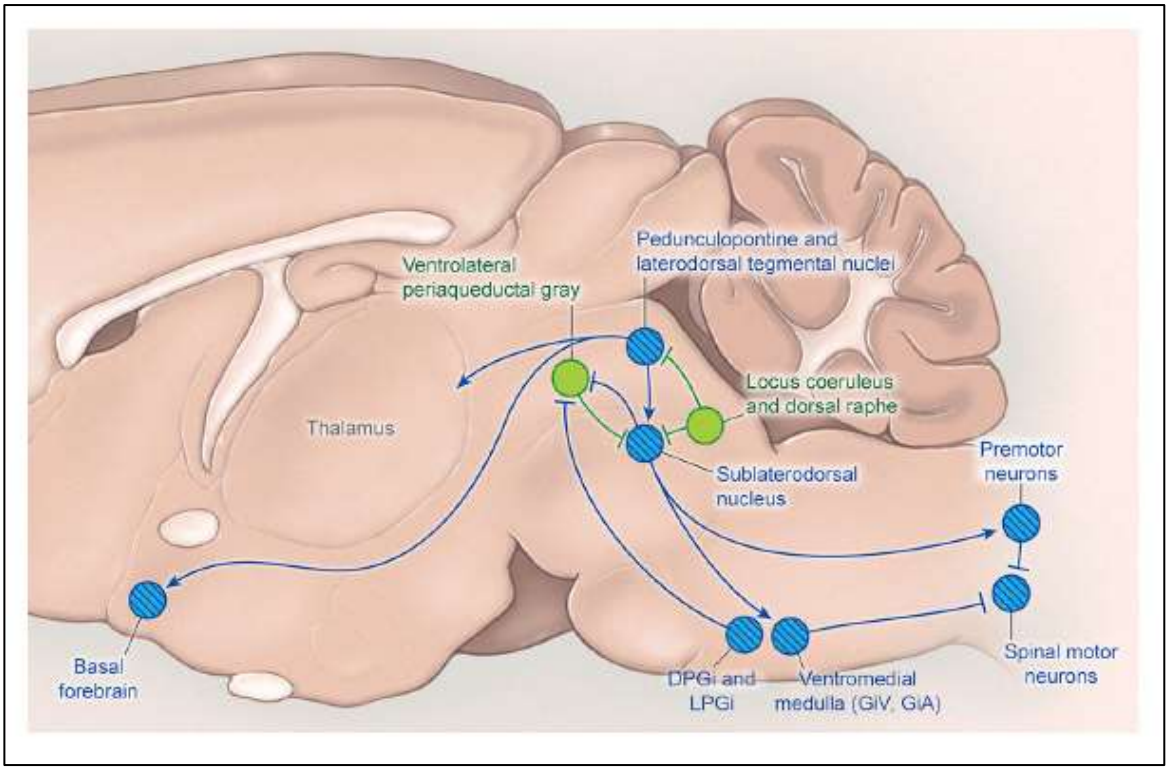


Figure 2.2 REM sleep promoting pathways (blue) and REM sleep suppressing pathways (green). During REM sleep, pedunculopontine and laterodorsal tegmental nuclei are involved in the firing of cholinergic neurons that stimulate REM sleep and fasten brain waves. GABAergic neurons of SLD and medulla inhibit the activity of ventrolateral periaqueductal gray. During non-REM sleep, GABAergic neurons of the ventrolateral periaqueductal gray and adjacent lateral pontine tegmentum, and monoaminergic neurons of the locus coeruleus and raphe nuclei inhibit the activity of SLD. Adapted from Scammell et al. (2017).

DPGi: dorsal paragigantocellular reticular nucleus; LPGi: lateral paragigantocellular nucleus; GiV: ventral gigantocellular reticular nucleus; GiA: alpha gigantocellular reticular nucleus.

2.2 Pain

2.2.1 Definition of pain

The definition of pain is “an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” in the International Association for the Study of Pain (International Association for the Study of Pain., 2020). Pain is a symptom of underlying conditions such as tissue injury, fibromyalgia and painful diabetic neuropathy.

2.2.2 Nociceptor

A nociceptor is a pain receptor that responds selectively to noxious stimuli in the form of thermal, chemical and mechanical (Gold and Gebhart, 2010). Nociceptors can be found in the periphery (skin) and the visceral organs. The nociceptors at the peripheral nerve endings are formed by primary afferent Aδ and C fibers (Reddi et al., 2013). Aδ is a myelinated fiber, whereas C-fiber is unmyelinated. The nodes of Ranvier present in the myelinated fibers facilitated the propagation of impulses and increased the conduction velocity of impulses (Yam et al., 2018).

2.2.3 Neurotransmitter in the transmission of pain

Neurotransmitter is a chemical messenger released by one neuron at a synapse. The neurotransmitter will bind to postsynaptic receptors of the second neuron and produce changes to the postsynaptic membrane potential. There are two types of neurotransmitters, which are known as excitatory neurotransmitter and inhibitory neurotransmitter. The excitatory neurotransmitters are involved in conducting signals for the transmission of pain impulses. Inhibitory neurotransmitters, on the other hand, may

inhibit the process of pain transmission. Chemical neurotransmitters involved in pain transmission are varies, as listed in Table 2.1 (Steeds, 2016).

Table 2.1 The classification of neurotransmitters

Class	Neurotransmitters	
	Excitatory	Inhibitory
Amines	• Adenosine triphosphate (ATP)	• Noradrenaline
Endogenous opioid peptides	• None	• Enkephalins
Non-opioid peptides	• Substances P	• Galanin
Amino acids	• Glutamate	• Gamma aminobutyric acid (GABA)

2.2.4 Pain pathways

The major pain pathways are the spinothalamic and spinoreticular tracts. The first pathway is the spinothalamic tract (Figure 2.3). The spinothalamic tract carries information from primary afferent fibers, which synapse with the second-order neurons in the dorsal grey horn of the spinal cord. The second-order neurons cross to the opposite side two segments above the level of entry via the anterior white commissure and ascend upwards to the thalamus. In the thalamus, the second-order neurons synapse with third-order neurons. The third-order neurons project and terminate at the somatosensory cortex. The neurons project to various brain regions, including the prefrontal cortex, somatosensory cortices and cingulate cortex for pain perception before interacting with the basal ganglia and cerebellum that are important for motor function. The spinothalamic tract also projects to the brainstem, hypothalamus and limbic system (Al-Chalabi and Reddy, 2020).

The second pathway is the spinoreticular tract. The spinoreticular tract consists of fibers that travel through the reticular formation, formed by the midbrain, pons and medulla, before ascending to the ventral posterior nuclei of the thalamus and the cerebral cortex (Almeida et al., 2004; Swenson, 2006). The spinoreticular tract of pain pathways plays an important role in emotionally disturbing pain (Fregoso et al., 2019). Projection to the reticular formation will activate periaqueductal gray (PAG), which form descending pathways. These pathways are involved in the modulation of pain transmission (Mokhtar and Singh, 2020).

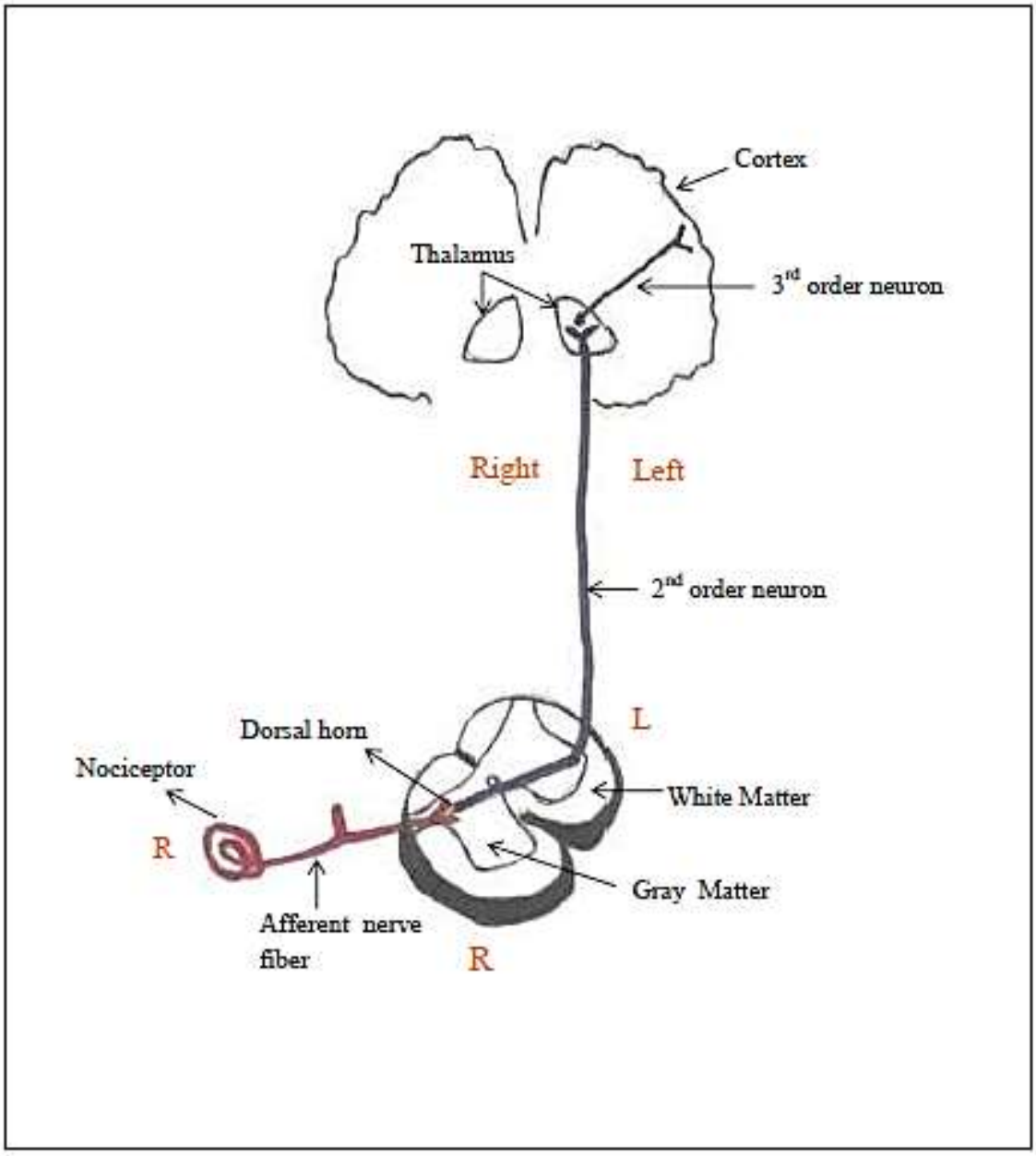


Figure 2.3 Spinothalamic tract of the pain pathway. Modified from Dudley (2021).

2.2.5 Modulation of pain

Pain information has to undergo various processes, including transduction, transmission and modulation, before it can be perceived (Sharma and Das, 2018), as shown in Figure 2.4. Transduction of pain occurs with activation of nociceptors due to stimulation by noxious stimuli, while transmission of pain information occurs through the peripheral afferent fibers and ascending pain pathways. The neurons project to various brain regions, including the prefrontal cortex, somatosensory cortices and cingulate cortex for pain perception.

Modulation of pain information can occur in the periphery and central levels. Pain modulation may result in inhibition or facilitation of impulse transmission (Steeds, 2016; Yam et al., 2018). At the periphery, reduced threshold of nociception and augmented response of afferent nerve fibers refers to peripheral sensitisation that leads to primary hyperalgesia, which means increased stimulus-dependent pain. Peripheral sensitisation can occur in the presence of tissue/nerve injury or inflammation. The release of neurotransmitters and endogenous chemicals from peripheral nerve endings and injured tissue will activate peripheral sensitisation. Bradykinins, prostaglandins and extracellular ATP are the pro-nociceptive mediators involved in nociception (Wei et al., 2020).

Meanwhile, at the spinal cord, segmental inhibition and descending inhibition may reduce the transmission of pain information. Segmental inhibition can be performed by the inhibitory interneurons that inhibit the conduction of impulses (Steeds, 2016). Gate control theory is another proposed mechanism that inhibits the conduction of impulses to the brain (Moayedi and Davis, 2013; Solepure, 2020).

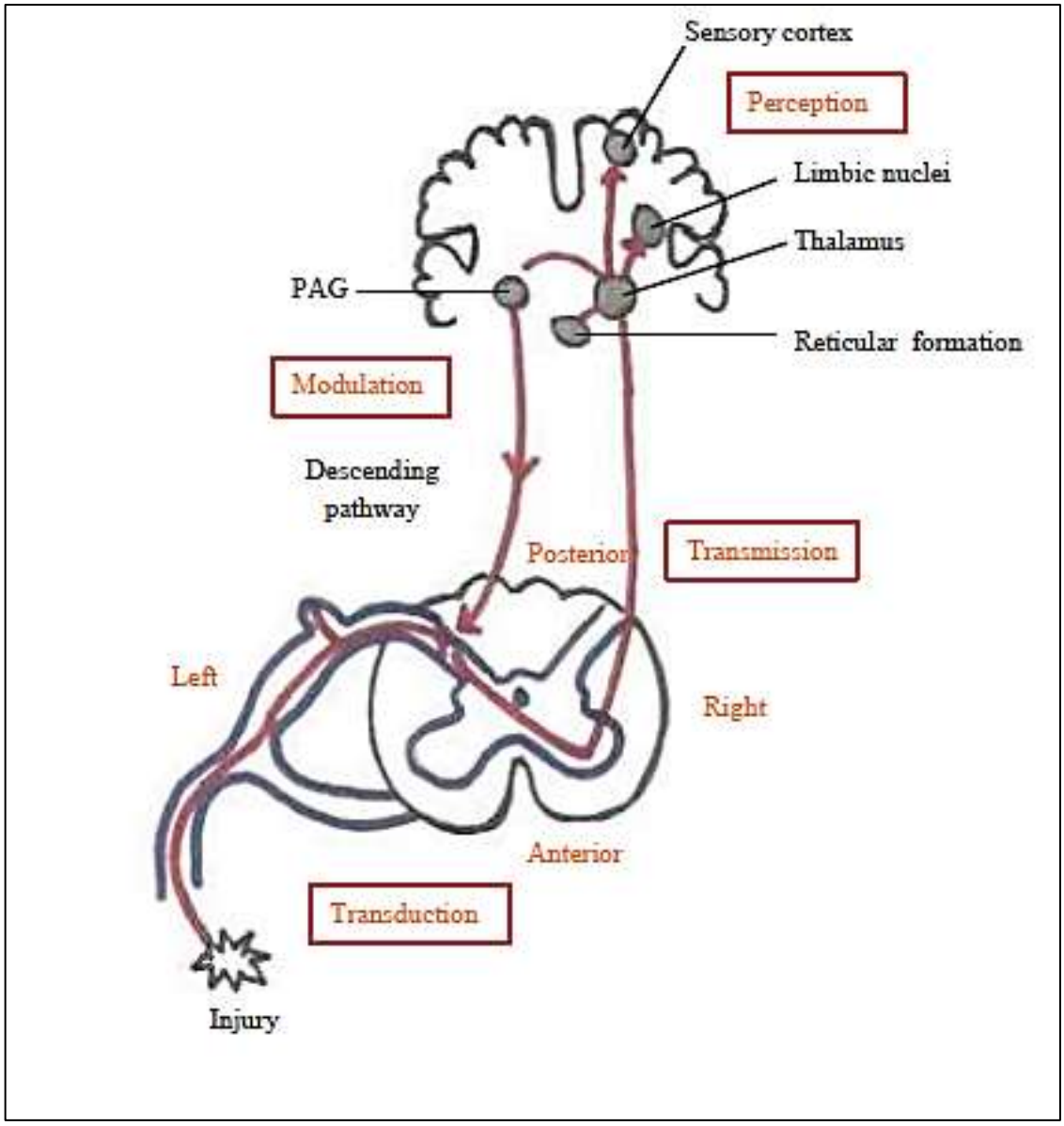


Figure 2.4 The transduction, transmission, modulation and perception of pain pathways. Adapted from Sharma and Das (2018).

2.2.5(a) Gate control theory

Melzack and Wall proposed Gate control theory of pain in 1965, as shown in Figure 2.5. There are two regions of cells in the dorsal horn of the spinal cord that are involved in pain transmission, which are the cells of substantia gelatinosa and "transmission" cells. The transmission of sensory signals from primary afferent neurons through substantia gelatinosa that acts as a gate to the transmission cells in the spinal cord. Activities of small and large fibers control the opening and closing of the gate. Activation of large fibers such as Aβ fibers stimulates the closing of the gate. Meanwhile, activation of small fibers such as C fibers stimulates the opening of the gate. The gate "opens" when the signal elicited is above the threshold and activates pain and its related behaviour. If the large and small fibers are activated simultaneously, the gate will be closed. Although both Aβ and C fibers stimulate transmission cells, impulses from Aβ will activate the inhibitory interneurons leading to the closure of the gate (Moayedi and Davis, 2013; Solepure, 2020).

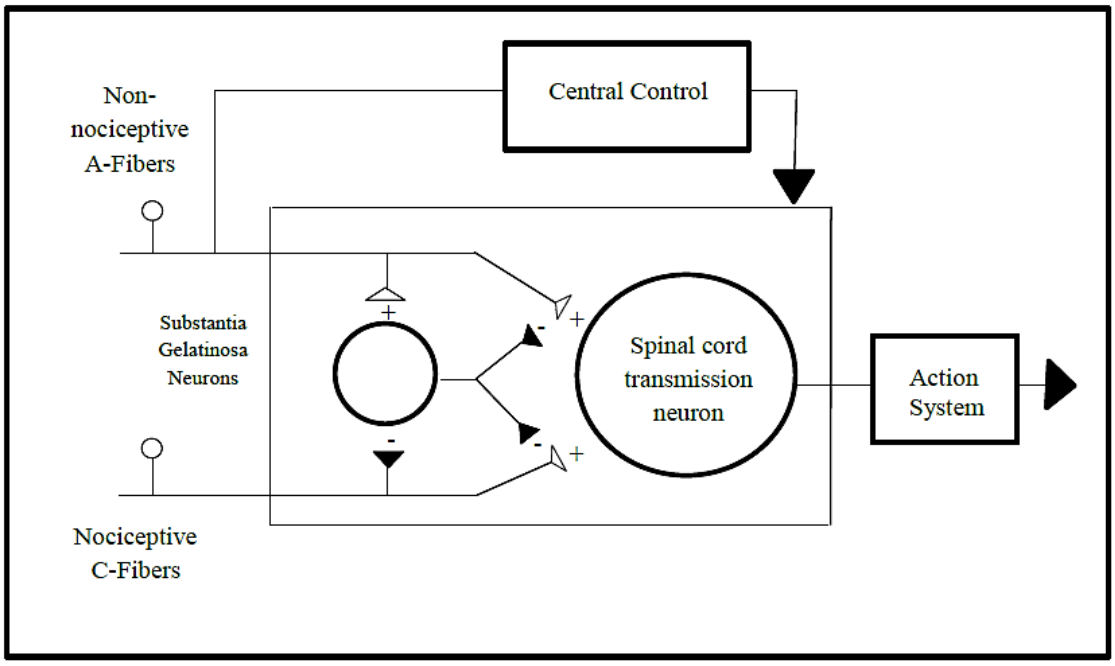


Figure 2.5 Gate control theory by Melzack and Wall. The activation of non-nociceptive A-fiber stimulates substantia gelatinosa neurons that inhibit synaptic transmission resulting in inhibition of pain transmission. However, the activation of nociceptive C-fiber inhibits substantia gelatinosa neurons and stimulates synaptic transmission resulting in pain transmission. Modified from Sufka and Price (2002).

2.2.5(b) Descending inhibition

The periaqueductal gray (PAG) is the brain region involved in pain modulation. The ascending pain pathways also project to the PAG. When the ascending pathways transmit the pain information, PAG will be activated. Descending inhibition by PAG is related to its connection with rostroventromedial medulla (RVM). When RVM receives dense input from PAG, it conveys the signal through the descending fibers that terminate in the dorsal horn of the spinal cord. PAG-RVM has a close relationship and the most studied circuit in descending pain modulation (Chen and Heinricher, 2019), as shown in Figure 2.6. The neurotransmitters in descending inhibition pathway are noradrenaline, 5-hydroxytryptamine (5HT), serotonin, dopamine, enkephalins and B-endorphin. Descending pathways inhibit the transmission of pain by activating inhibitory interneurons in the spinal cord (Squire et al., 2003) and inhibiting nociceptive neurons in the dorsal horn (Ossipov et al., 2010; Bannister and Dickenson, 2016).

2.2.6 Sensitisation of pain

2.2.6(a) Central sensitisation

Central sensitisation occurs with repeated stimulation of C fibers that lead to a prolonged response of the dorsal horn after nerve injury. Central sensitisation also increased membrane excitability and synaptic efficacy in pain transmission. Changes in neuronal signalling, such as activation of the N-methyl-D-aspartate (NMDA) receptor and release of nitric oxide and neurokinins in the dorsal horn despite local inhibition by GABA and glycine, resulted in a reduction in pain signalling threshold. The persistent C-fiber nociceptors firing leads to the release of glutamate, which acts on NMDA receptors in

the spinal cord and unblocks magnesium ions from the NMDA receptor (Bennett, 2000). There are also changes in the structural rewiring in the dorsal horn of the spinal cord, including the degeneration of C-fiber and sprout of Aβ (fine touch) fiber in substantia gelatinosa (lamina II). Sprouting of Aβ to lamina II leads to access to C fibers leading to the activation of substance P and excitatory amino acids that bind to neurokinin-1 (NK1) and NMDA receptors that cause wind up phenomena that increase in the excitability of spinal cord neurons (Hoseini et al., 2006). The structural changes of the dorsal horn and loss of nerve growth factor lead to allodynia in patients (Latremoliere and Woolf, 2009; Steeds, 2016). Central sensitisation also reduced the activity of descending modulation, contributing to enhanced pain responses (Latremoliere and Woolf, 2009).

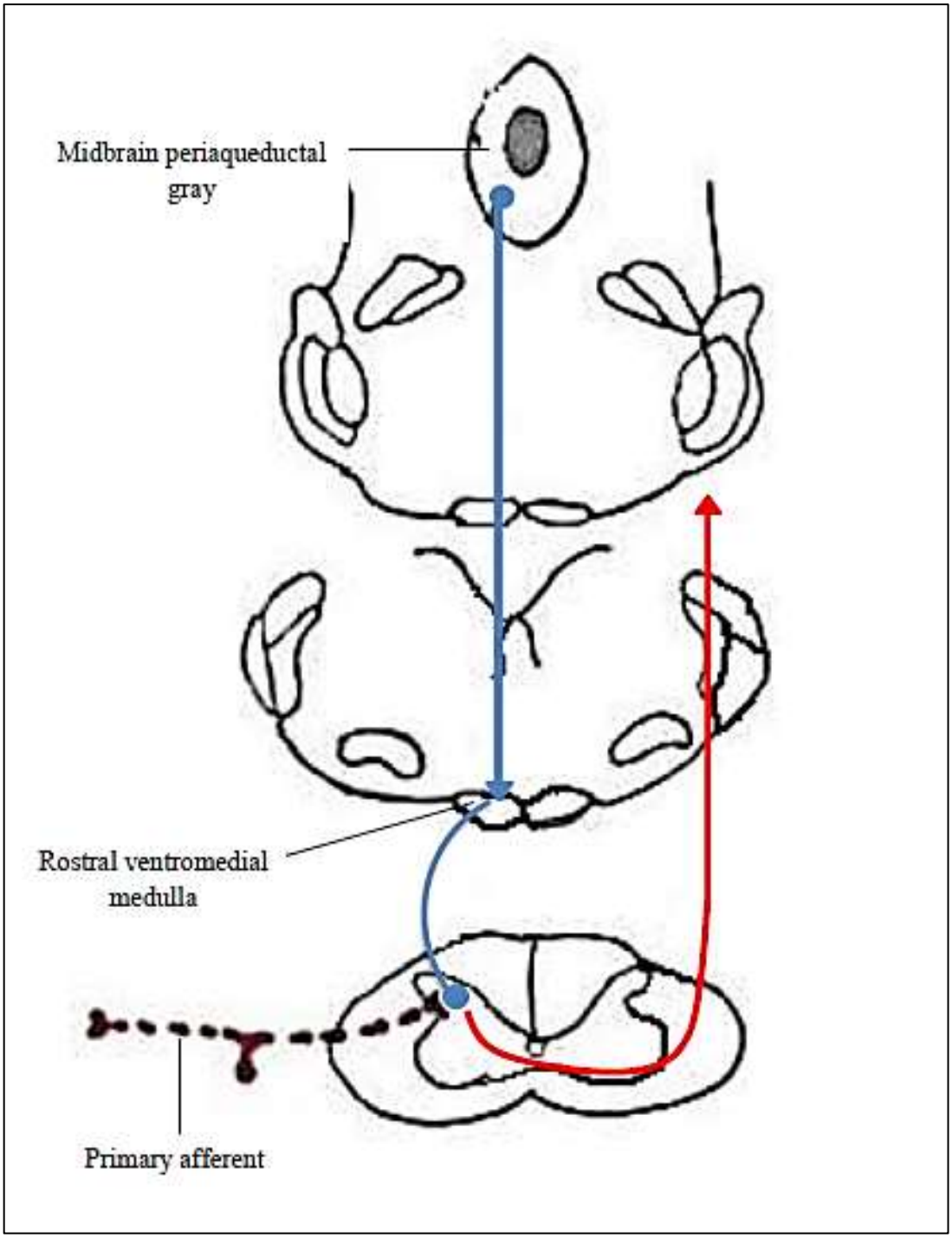


Figure 2.6 The modulation of pain between periaqueductal grey and rostroventromedial medulla (PAG-RVM). Modified from Chen and Heinricher (2019).

* Ascending pathways (example: Spinothalamic and spinoreticular tract)

Descending modulation (example: gate control theory and descending inhibition)

2.2.7 Animal pain models

There are multiple animal pain models and the selected model in an experiment must be appropriate depending on the research objectives. Pain in the animals is assessed by observing behavioural responses following stimulation of the animal with a chosen stimulus. The pain tests are categorised into reflexive and non-reflexive tests. Examples of reflexive tests are tail flick test and hot plate test in which thermal stimulus is applied to the animals. Meanwhile, the non-reflexive test is spontaneous pain behaviour observed after chemical stimulation, such as injecting the animal with formalin, carrageenan, or acetic acid (Gregory et al., 2013). Primary hyperalgesia is indicated by changes in threshold and responses to stimuli. Meanwhile, secondary hyperalgesia is indicated by the changes outside the site of application or injury (Gregory et al., 2013).

2.2.7(a) Reflexive pain tests

In this type of test, pain is triggered by applying heat, cold, or mechanical stimuli. This test activates the nociceptors in the stimulated area and produces a localised pain response.

2.2.7(a)(i) Tail flick test

The earliest test of pain was introduced by D'Amour and Smith in 1941, which was the tail flick test. In the tail flick test, the rat's tail was placed on a hot plate and the time taken for the rats to avoid the stimuli was measured (Gregory et al., 2013). However, the tail flick test can be applied with the interval of five minutes rest between each evaluation. The tail flick test was a commonly used nociceptive test in rodents (Le Bars et al., 2001).

Erejuwa, O. O., Sulaiman, S. A., Wahab, M. S., Sirajudeen, K. N. S., Salleh, M. S. M. D., & Gurtu, S. (2010). Antioxidant protection of Malaysian tualang honey in pancreas of normal and streptozotocin-induced diabetic rats. *Annales d'Endocrinologie*, **71**(**4**), 291–296. doi:10.1016/j.ando.2010.03.003

Estabrooke, I. V, Mccarthy, M. T., Ko, E., Chou, T. C., Chemelli, R. M., Yanagisawa, M., … Scammell, T. E. (2001). Fos Expression in Orexin Neurons Varies with Behavioral State. *The Journal of Neuroscience*, **21**(**5**), 1656–1662.

Everson, C. A., Laatsch, C. D., & Hogg, N. (2005). Antioxidant defense responses to sleep loss and sleep recovery. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology*, **288**, 374–383. doi:10.1152/ajpregu.00565.2004

Everson, C. A., & Szabo, A. (2011). Repeated exposure to severely limited sleep results in distinctive and persistent physiological imbalances in rats. *PLoS ONE*, **6**(**8**). doi:10.1371/journal.pone.0022987

Fehrenbacher, J. C., Vasko, M. R., & Duarte, D. B. (2012). Models of Inflammation: Carrageenan- or Complete Freund's Adjuvant-Induced Edema and Hypersensitivity in the Rat. *Current Protocol of Pharmacology*, **56**(**1**), 5.4.1-5.4.4. doi:10.1002/0471141755.ph0504s56

Finaud, J., Lac, G., & Filaire, E. (2006). Oxidative Stress Relationship with Exercise and Training. *Sports Medicine*, **36**(**4**), 327–358.

Fox, C. J., Russell, K. I., Wang, Y. T., & Christie, B. R. (2006). Contribution of NR2A and NR2B NMDA Subunits to Bidirectional Synaptic Plasticity in the Hippocampus In Vivo. *Hippocampus*, **16**, 907–915. doi:10.1002/hipo

Foo, H., & Mason, P. (2003). Brainstem modulation of pain during sleep and waking. *Sleep Medicine Reviews*, **7**(**2**), 145–154. doi:10.1053/smrv.2002.0224

Francis, J. T., Xu, S., & Chapin, J. K. (2008). Proprioceptive and cutaneous representations in the rat ventral posterolateral thalamus. *Journal of Neurophysiology*, **99**(**5**), 2291–2304. doi:10.1152/jn.01206.2007

Fregoso, G., Wang, A., Tseng, K., & Wang, J. (2019). Transition from Acute to Chronic Pain: Evaluating Risk for Chronic Postsurgical Pain. *Pain Physician*, **22**, 479–488.

Gao, X., Kim, H. K., Chung, J. M., & Chung, K. (2007). Reactive oxygen species (ROS) are involved in enhancement of NMDA-receptor phosphorylation in animal models of pain. *Pain*, **131**, 262–271. doi:10.1016/j.pain.2007.01.011

Gaucher, C., Boudier, A., Bonetti, J., Clarot, I., Leroy, P., & Parent, M. (2018). Glutathione : Antioxidant Properties Dedicated to to Nanotechnologies Nanotechnologies. *Antioxidants*, **7**(**62**). doi:10.3390/antiox7050062

Gawade, S. P. (2012). Acetic acid induced painful endogenous infliction in writhing test on mice. *Journal of Pharmacology and Pharmacotherapeutics*, **3**(**4**), 348. doi:10.4103/0976-500X.103699

Goettl, V. M., Huang, Y., Hackshaw, K. V, & Stephens, R. L. (2002). Reduced basal release of serotonin from the ventrobasal thalamus of the rat in a model of neuropathic pain. *Pain*, **99**, 359–366. doi:https://doi.org/10.1016/S0304-3959(02)00209-9

Gold, M. S., & Gebhart, G. F. (2010). HHS Public Access. *Nature Medicine*, **16**(**11**), 1248–1257. doi:10.1038/nm.2235.Nociceptor

Gopalakrishnan, A., Ji, L. L., & Cirelli, C. (2004). Sleep deprivation and cellular responses to oxidative stress. *Sleep*, **27**(**1**), 27–35. doi:10.1093/sleep/27.1.27

Gregory, N., Harris, A., Dugherty, P., Fuchs, P., & Sulka, K. A. (2013). An overview of animal models of pain: disease models and outcome measures. *Journal of Pain*, **14**(**11**), 1–26. doi:10.1016/j.jpain.2013.06.008

Groh, A., Mease, R. A., & Krieger, P. (2017). Pain processing in the thalamocortical system. *Neuroforum*, **23**(**3**), 117–122. doi:10.1515/nf-2017-A019

Guilbaud, G., Benoist, J. M., Jayser, V., & Gautron, M. (1987). Neuronal response thresholds to and encoding of thermal stimuli during carrageenin-hyperalgesic-inflammation in the ventro-basal thalamus of the rat. *Experimental Brain Research*, **66**, 421–431.

Guilbaud, G., Kayser, V., Benoist, J. M., & Gautron, M. (1986). Modifications in the Responsiveness of Rat Ventrobasal Thalamic Neurons at Different Stages of Carrageenin-Produced Inflammation. *Brain Research*, **385**, 86–98. doi:https://doi.org/10.1016/0006-8993(86)91550-7

Guilbaud, G., Peschanski, M., Gautron, M., & Binder, D. (1980). Neurones responding to noxious stimulation in the VB complex and caudal adjacent regions in the thalamus of the rat. *Pain*, **8**, 303–318. doi:https://doi.org/10.1016/0304-3959(80)90076-7

Gunn, A., Bobeck, E. N., Weber, C., & Morgan, M. M. (2011). The Influence of Non-Nociceptive Factors on Hot Plate Latency in Rats. *Journal of Pain*, **12**(**2**), 222–227. doi:10.1016/j.jpain.2010.06.011

Guo, Y. P., Zhi, Y. R., Liu, T. T., Wang, Y., & Zhang, Y. (2019). Global gene knockout of kcnip3 enhances pain sensitivity and exacerbates negative emotions in rats. *Frontiers in Molecular Neuroscience*, **12**. doi:10.3389/fnmol.2019.00005

Gustin, S. M., Wrigley, P. J., Youssef, A. M., McIndoe, L., Wilcox, S. L., Rae, C. D., … Henderson, L. A. (2014). Thalamic activity and biochemical changes in individuals with neuropathic pain following spinal cord injury. *Pain*, **155**(**5**), 1027–1036. doi:10.1016/j.pain.2014.02.008

Hasim, H., Abd Aziz, C. B., Ahmad Suhaimi, S. Q., & Mohamed, M. (2020). Effects of Tualang Honey on Pain Behaviour and Oxidative Stress in the Thalamus of Prenatally Stressed Rat Offspring. *Malaysian Journal of Medicine and Health Sciences*, **16**(**3**), 85–92.

Haack, M., Simpson, N., Sethna, N., Kaur, S., & Mullington, J. (2020). Sleep deficiency and chronic pain: potential underlying mechanisms and clinical implications. *Neuropsychopharmacology*, **45**(**1**), 205–216. doi:10.1038/s41386-019-0439-z

Hanlon, E. C., Andrzejewski, M. E., Harder, B. K., Kelley, A. E., & Benca, R. M. (2005). The effect of REM sleep deprivation on motivation for food reward. *Behavioural Brain Research*, **163**(**1**), 58–69. doi:10.1016/j.bbr.2005.04.017

Hasim, H., Abd Aziz, C. B., Ahmad Suhaimi, S. Q., & Mohamed, M. (2020). Effects of Tualang Honey on Pain Behaviour and Oxidative Stress in the Thalamus of Prenatally Stressed Rat Offspring. *Malaysian Journal of Medicine and Health Sciences*, **16**(**3**), 85–92.

Havekes, R., Park, A. J., Tudor, J. C., Luczak, V. G., Hansen, R. T., Ferri, S. L., … Abel, T. (2016). Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. *ELife*, **5**, 1–22. doi:10.7554/elife.13424

Hayati, A. A., Zalina, I., Myo, T., Che Badariah, A. A., Azhar, A., & Idris, L. (2008). Modulation of formalin-induced fos-like immunoreactivity in the spinal cord by swim stress-induced analgesia, morphine and ketamine. *German Medical Science : GMS e-Journal*, **6**, Doc05. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19675733%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2703257

Heck, D. E., Shakarjian, M., Kim, H. D., Laskin, J. D., & Vetrano, A. M. (2010). Mechanisms of oxidant generation by catalase. *Annals of the New York Academy of Sciences*, **1203**, 120–125. doi:10.1111/j.1749-6632.2010.05603.x.

Higashi, Y., Aratake, T., Shimizu, T., Shimizu, S., & Saito, M. (2021). Protective role of glutathione in the hippocampus after brain ischemia. *International Journal of Molecular Sciences*, **22**(**15**). doi:10.3390/ijms22157765

Hipólide, D. C., Suchecki, D., de Carvalho Pinto, A. P., Chiconelli Faria, E., Tufik, S., & Luz, J. (2006). Paradoxical sleep deprivation and sleep recovery: Effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats. *Journal of Neuroendocrinology*, **18**(**4**), 231–238. doi:10.1111/j.1365-2826.2006.01412.x

Hendrix, J., Nijs, J., Ickmans, K., Godderis, L., Ghosh, M., & Polli, A. (2020). The Interplay between Oxidative Stress, Exercise , and Pain in Health and Disease : Potential Role of Autonomic Regulation and Epigenetic Mechanisms. *Antioxidants*, **9**(**1166**).

Hoseini, S. S., Hoseini, M., & Gharibzadeh, S. (2006). Sprouting phenomenon, a new model for the role of A-β fibers in wind up. *Medical Hypotheses*, **66**(**4**), 805–807. doi:10.1016/j.mehy.2005.09.047

Hu, J., Wang, Z., Guo, Y., Zhang, X., Xu, Z., Liu, S., … Zhao, M. (2009). A role of periaqueductal grey NR2B-containing NMDA receptor in mediating persistent inflammatory pain. *Molecular Pain*, **5**(**71**). doi:10.1186/1744-8069-5-71

Huang, W., Guo, B., Shen, Y., Tang, X., Zhang, T., & Li, D. (2020). Sleep staging algorithm based on multichannel data adding and multifeature screening. *Computer Methods and Programs in Biomedicine*, **187**, 105253. doi:10.1016/j.cmpb.2019.105253

Hunt, D. L., & Castillo, P. E. (2012). functional implications. *Current Opinion in Neurobiology*, **22**(**3**), 496–508. doi:10.1016/j.conb.2012.01.007.Synaptic

Iacovides, S., George, K., Kamerman, P., & Baker, F. C. (2017). Sleep fragmentation hypersensitizes healthy young women to deep and superficial experimental pain. *Journal of Pain*, **18**, 844–854. doi:10.1016/j.jpain.2017.02.436

International Association for the Study of Pain. (2020). Revised Definition of Pain. Retrieved September 16, 2021, from https://www.iasp-pain.org/publications/iasp-news/iasp-announces-revised-definition-of-pain/

Ismail, C. A. N., Che Hussin, C. M., Mohamed, M., & Abd Aziz, C. B. (2017). Preemptive Effects of Administration of Tualang Honey on Inflammatory Responses in Adult Male Rats. *Journal of Pharmacy and Nutrition Sciences*, **7**, 6–12.

Jan, J. E., Reiter, R. J., Wasdell, M. B., & Martin, B. (2009). The role of the thalamus in sleep , pineal melatonin production , and circadian rhythm sleep disorders. *Journal of Pineal Research*, **46**, 1–7. doi:10.1111/j.1600-079X.2008.00628.x

Jung, T., & Noh, J. (2021). Alteration of fear behaviors in sleep-deprived adolescent rats : increased fear expression and delayed fear extinction. *Animal Cells and Systems*, **25**(**2**), 83–92. doi:10.1080/19768354.2021.1902854

Kamat, P. K., Kalani, A., Rai, S., Swarnkar, S., Tota, S., Nath, C., & Tyagi, N. (2016). Mechanism of Oxidative Stress and Synapse Dysfunction in the Pathogenesis of Alzheimer's Disease: Understanding the Therapeutics Strategies. *Molecular Neurobiology*, **53**(**1**), 648–661. doi:10.1007/s12035-014-9053-6

Khalid, A. F., Tan, J. J., & Yong, Y. K. (2018). Malaysian Tualang Honey and Its Potential Anti-Cancer Properties : A Review. *Sains Malaysiana*, **47**(**11**), 2705–2711.

Khalil, M. I., Sulaiman, A. A., Ramli, N., Mohamed, M., Bai'e, S., & Hua, G. S. (2012). Content and antioxidant properties of processed Tualang Honey (AgroMas) collected from different regions in Malaysia. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4**, 214–219.

Khandelwal, D., Dutta, D., Chittawar, S., & Kalra, S. (2017). Sleep disorders in type 2 diabetes. *Indian Journal of Endocrinology and Metabolism*, **21**(**5**), 758–761. doi:10.4103/ijem.IJEM_156_17

Kim, S. H., Park, J. Y., Shin, H. E., Lee, S. B., Ryu, D. W., Kim, T. W., & Park, J. W. (2019). The influence of rapid eye movement sleep deprivation on nociceptive transmission and the duration of facial allodynia in rats: a behavioral and Fos immunohistochemical study. *The Journal of Headache and Pain*, **20**(**21**), 1–9.

Kishore, R. K., Sukari, A., Syazana, M. S. N., & Sirajudeen, K. N. S. (2011). Tualang honey has higher phenolic content and greater radical scavenging activity compared with other honey sources. *Nutrition Research*, **31**, 322–325. doi:10.1016/j.nutres.2011.03.001

Koban, M., Sita, L. V, Le, W. W., & Hoffman, G. E. (2008). Sleep Deprivation of Rats : The Hyperphagic Response Is Real. *Sleep*, **31**(**7**), 1–7.

Koban, M., & Swinson, K. L. (2005). Chronic REM-sleep deprivation of rats elevates metabolic rate and increases UCP1 gene expression in brown adipose tissue. *American Journal of Physiology - Endocrinology and Metabolism*, **289**, 68–74. doi:10.1152/ajpendo.00543.2004

Kolhekar, R., Murphy, S., & Gebhart, G. F. (1997). Thalamic NMDA receptors modulate inflammation-produced hyperalgesia in the rat. *Pain*, **71**, 31–40.

Kopp, C., Longordo, F., Nicholson, J. R., & Lüthi, A. (2006). Insufficient sleep reversibly alters bidirectional synaptic plasticity and NMDA receptor function. *Journal of Neuroscience*, **26**(**48**), 12456–12465. doi:10.1523/JNEUROSCI.2702-06.2006

Kumar, K., Taylor, R. S., Jacques, L., Eldabe, S., Meglio, M., Molet, J., … North, R. B. (2007). Spinal cord stimulation versus conventional medical management for neuropathic pain: A multicentre randomised controlled trial in patients with failed back surgery syndrome. *Pain*, **132**, 179–188. doi:10.1016/j.pain.2007.07.028

Latremoliere, A., & Woolf, C. J. (2009). Central Sensitization: A Generator of Pain Hypersensitivity by Central Neural Plasticity. *Journal of Pain*, **10**(**9**), 895–926. doi:10.1016/j.jpain.2009.06.012.

Lautenbacher, S., Kundermann, B., & Krieg, J.-C. (2006). Sleep deprivation and pain perception. *Sleep Medicine*, **10**, 357–369. doi:10.1016/j.smrv.2005.08.001

Le Bars, D., Gozariu, M., & Cadden, S. W. (2001). Evaluation de la douleur aiguë chez l'animal d'expérience. Première partie [Acute pain measurement in animals Part 1]. *Annales Françaises d'Anesthésie et de Réanimation*, **20**(**4**), 347–365. doi:10.1016/s0750-7658(01)00381-1

Lee, I.-O., & Crosby, G. (1999). Halothene effect on formalin-induced paw edema and flinching in rat. *Journal of Korean Medical Science*, **14**, 34–38. doi:10.3346/jkms.1999.14.1.34

Lenz, F. A., Weiss, N., Ohara, S., Lawson, C., & Greenspan, J. D. (2004). Chapter 6 The role of the thalamus in pain. *Supplements to Clinical Neurophysiology*, **57**, 50–61. doi:10.1016/S1567-424X(09)70342-3

Li, C., Li, J., Jiang, Y., Mu, Y., Lu, D., Xiao, Z., … Chen, X. (2020a). Decreased cpg15 augments oxidative stress in sleep deprived mouse brain. *Biochemical and Biophysical Research Communications*, **522**(**3**), 749–756. doi:10.1016/j.bbrc.2019.11.132

Li, L., Gan, Y., Zhou, X., Jiang, H., Zhao, Y., Tian, Q., … Lu, Z. (2020b). Insomnia and the risk of hypertension : A meta-analysis of prospective cohort studies. *Sleep Medicine Reviews*, 56, 101403. doi:10.1016/j.smrv.2020.101403

Li, T.-T., Ren, W.-H., Xiao, X., Nan, J., Cheng, L.-Z., Zhang, X.-H., … Zhang, Y.-Q. (2009). NMDA NR2A and NR2B Receptors in the Rostral Anterior Cingulate Cortex Contribute to Pain-related Aversion in Male Rats. *Pain*, **146**(**1–2**), 183–193. doi:10.1016/j.pain.2009.07.027

Li, V., & Wang, Y. T. (2016). Molecular mechanisms of NMDA receptor-mediated excitotoxicity: Implications for neuroprotective therapeutics for stroke. *Neural Regeneration Research*, **11**(**11**), 1752–1753. doi:10.4103/1673-5374.194713

Liu, C., Kong, X., Liu, R., & Wu, B. (2013). Long-term total sleep deprivation reduces thalamic gray matter volume in healthy men matter volume in healthy men. *Neuroreport*, **25**, 320–323. doi:10.1097/WNR.0000000000000091

Liu, S., Shen, J., Li, Y., Wang, J., Wang, J., Xu, J., … Chen, R. (2021). EEG Power Spectral Analysis of Abnormal Cortical Activations During REM / NREM Sleep in Obstructive Sleep Apnea. *Frontiers in Neurology*, **12**, 180. doi:10.3389/fneur.2021.643855

Ma, N., Dinges, D. F., Basner, M., & Rao, H. (2015). How acute total sleep loss affects the attending brain: A meta-analysis of neuroimaging studies. *Sleep*, **38**(**2**), 233–240. doi:10.5665/sleep.4404

Ma, Z., Liu, K., Li, X., Wang, C., Liu, C., Yan, D., … Xu, B. (2020). Alpha-synuclein is involved in manganese-induced spatial memory and synaptic plasticity impairments via TrkB/Akt/Fyn-mediated phosphorylation of NMDA receptors. *Cell Death and Disease*, **11**, 834. doi:10.1038/s41419-020-03051-2

Maloney, K. J., Mainville, L., & Jones, B. E. (1999). Differential c-Fos expression in cholinergic, monoaminergic, and GABAergic cell groups of the pontomesencephalic tegmentum after paradoxical sleep deprivation and recovery. *Journal of Neuroscience*, **19**(**8**), 3057–3072. doi:10.1523/jneurosci.19-08-03057.1999

Maneshi, M. M., Maki, B., Gnanasambandam, R., Belin, S., Popescu, G. K., Sachs, F., & Hua, S. Z. (2017). Mechanical stress activates NMDA receptors in the absence of agonists. *Scientific Reports*, **7**(**1**), 1–10. doi:10.1038/srep39610

Mathangi, D. C., Shyamala, R., & Subhashini, A. S. (2012). Effect of REM sleep deprivation on the antioxidant status in the brain of Wistar rats. *Annals of Neurosciences*, **19**(**4**), 161–164.

May, J. M. (2012). Vitamin C transport and its role in the central nervous system. *Sub-Cellular Biochemistry*, **56**, 85–103. doi:10.1007/978-94-007-2199-9_6

May, M. E., Harvey, M. T., Valdovinos, M. G., Kline IV, R. H., Wiley, R. G., & Kennedy, C. H. (2005). Nociceptor and age specific effects of REM sleep deprivation induced hyperalgesia. *Behavioural Brain Research*, **159**(**1**), 89–94. doi:10.1016/j.bbr.2004.10.005

Metz, A. E., Yau, H.-J., Centeno, M. V., Apkarian, V. A., & Martina, M. (2009). Morphological and functional reorganization of rat medial prefrontal cortex in neuropathic pain. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(**7**), 2423–2428. doi:10.18848/1447-9516/cgp/v08i04/36903

Mcdermott, C. M., Hardy, M. N., Bazan, N. G., & Magee, J. C. (2006). Sleep deprivation-induced alterations in excitatory synaptic transmission in the CA1 region of the rat hippocampus. *J Physiology*, **3**(**570**), 553–565. doi:10.1113/jphysiol.2005.093781

McNamara, C. R., Mandel-Brehm, J., Bautista, D. M., Siemens, J., Deranian, K. L., Zhao, M., … Fanger, C. M. (2007). TRPA1 mediates formalin-induced pain. *Proceedings of the National Academy of Sciences of the United States of America*, **104**(**33**), 13525–13530. doi:10.1073/pnas.0705924104

McRoberts, J. A., Coutinho, S. V, Marvizo, J. C. G., Grady, E. F., Tognetto, M., Sengupta, J. N., … Mayer, E. A. (2001). Role of Peripheral N-Methyl-D-Aspartate (NMDA) Receptors in Visceral Nociception in Rats. *Gastroenterology*, **120**, 1737–1748. doi:10.1053/gast.2001.24848

Medic, G., Wille, M., & Hemels, M. E. H. (2017). Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, **9**, 151–161.

Miller, K. E., Hoffman, E. M., Sutharshan, M., & Schechter, R. (2011). Glutamate pharmacology and metabolism in peripheral primary afferents: Physiological and pathophysiological mechanisms. *Pharmocology & Therapeutics*, **130**(**3**), 283–309. doi:10.1016/j.pharmthera.2011.01.005.

Mir, F. A., Jha, S. K., & Jha, V. M. (2019). The Role of Sleep in Homeostatic Regulation of Ionic Balances and Its Implication in Cognitive Functions. In *Sleep, Memory and Synaptic Plasticity* (pp. 77–106). Singapore: Springer. doi:10.1007/978-981-13-2814-5

Moayedi, M., & Davis, K. D. (2013). Theories of pain : from specificity to gate control. *Journal of Neurophysiology*, **109**(**1**), 5–12. doi:10.1152/jn.00457.2012

Mohamad Shah, N. S., Gan, S. H., & Ahmad Sukari, H. (2013). Analysis of volatile compounds of malaysian Tualang (Kompassia excelsa) honey using gas chromatography mass spectrometry. *African Journal of Traditional and Complementary Alternative Medicine*, **10**(**2**), 180–188.

Mohamed, M., Sirajudeen, K. N. ., Swamy, M., Yaacob, N. S., & Sulaiman, S. A. (2010). Studies on the antioxidant properties of Tualang honey of Malaysia. *African Journal of Traditional, Complementary and Alternative Medicines*, **7**(**1**), 59–63.

Mohd Sairazi, N. S., Sirajudeen, K. N. S., Muzaimi, M., Mummedy, S., Asari, M. A., & Sulaiman, S. A. (2018). Tualang Honey Reduced Neuroinflammation and Caspase-3 Activity in Rat Brain after Kainic Acid-Induced Status Epilepticus. *Evidence-Based Complementary and Alternative Medicine*, **2018**. doi:10.1155/2018/7287820

Mohmed Nor, N. S., Nawi, A., Wan Ahmad, W. A. N., & Noordin, L. (2021). Sleep Deprivation Models in Rodents. *Jurnal Sains Kesihatan Malaysia*, **19**(**2**), 1–6.

Mokhtar, M., & Singh, P. (2020). Neuroanatomy, Periaqueductal Gray. In *StatPearls (Internet)*. StatPearls Publishing, Treasure Island (FL).

Moniruzzaman, M., Khalil, M. I., Sulaiman, S. A., & Gan, S. H. (2013). Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*. *BMC Complementary and Alternatuve Medicine*, **13**(**43**).

Mork, P. J., & Nilsen, T. I. L. (2012). Sleep Problems and Risk of Fibromyalgia : Longitudinal Data on an Adult Female Population in Norway. *Arthritis & Rheumatism*, **64**(**1**), 281–284. doi:10.1002/art.33346

Moussa, Z., Judeh, Z. M. A., & Ahmed, S. A. (2019). Nonenzymatic Exogenous and Endogenous Antioxidants. *Free Radical Medicine and Biology*, 1–30. doi:10.5772/intechopen.87778

Moussa, Z., Judeh, Z. M. A., & Ahmed, S. A. (2019). Nonenzymatic Exogenous and Endogenous Antioxidants. In K. Das, S. Das, M. Shivanagouda Biradar, V. Bobbarala, & S. Subba Tata (Eds.), *Radical Medicine and Biology*. London, UK: InTechOpen. doi:10.5772/intechopen.87778

Mullington, J. M., Hinze-Selch, D., & Pollmächer, T. (2011). Mediators of inflammation and their interaction with sleep: relevance for chronic fatigue syndrome and related conditions, *Annals of the New York Academy of Sciences*, **933**, 201-10. doi: 10.1111/j.1749-6632.2001.tb05825.x

Nawi, A., Eu, K. L., Ahmad Faris, A. N., Wan Ahmad, W. A. N., & Noordin, L. (2020). Lipid peroxidation in the descending thoracic aorta of rats deprived of REM

sleep using the inverted flowerpot technique. *Experimental Physiology*, 1–9. doi:10.1113/EP088667

Newcomer, J. W., Brber, N. B., & Olney, J. W. (2000). NMDA receptor function, memory, and brain aging. *Dialogues in Clinical Neuroscience*, **2**(**3**), 219–232.

Noguchi, Y., Fukuda, K., Matsushima, A., Haishi, D., Hiroto, M., Kodera, Y., … Inada, Y. (1999). Inhibition of Df-protease associated with allergic diseases by polyphenol. *Journal of Agricultural and Food Chemistry*, **47**(**8**), 2969–2972. doi:10.1021/jf9812073

Onen, S. H., Alloui, A., Jourdan, D., Eschalier, A., & Dubray, C. (2001). Effects of rapid eye movement (REM) sleep deprivation on pain sensitivity in the rat. *Brain Research*, **900**, 261–267.

Ossipov, M. H., Dussor, G. O., Porreca, F., Ossipov, M. H., Dussor, G. O., & Porreca, F. (2010). Central modulation of pain. *The Journal of Clinical Investigation*, **120**(**11**), 3779–3787. doi:10.1172/JCI43766

Othman, Z., Zakaria, R., Hazlina, N., Hussain, N., Shafin, N., Al-rahbi, B., & Ahmad, A. H. (2015). Potential Role of Honey in Learning and Memory. *Medical Science*, **3**, 3–15. doi:10.3390/medsci3020003

Owen, J. E., & Veasey, S. C. (2020). Impact of sleep disturbances on neurodegeneration: Insight from studies in animal models. *Neurobiology of Disease*, **139**, 104820. doi:10.1016/j.nbd.2020.104820

Pandey, A., & Kar, S. K. (2018). Rapid Eye Movement sleep deprivation of rat generates ROS in the hepatocytes and makes them more susceptible to oxidative stress. *Sleep Science*, **11**(**4**), 245–253. doi:10.5935/1984-0063.20180039

Parameswari, P., Chethan, N., & Saravana Babu, C. (2017). Neurochemicals and Behavioural Alterations in Sleep Deprivation: A Review. *Journal of Dementia*, **1**(**1**), 104. Retrieved from https://www.omicsonline.org/open-access/neurochemicals-and-behavioural-alterations-in-sleep-deprivation-a-revisit-95632.html

Paxinos, G., & Watsons, C. (2004). *The rat brain in stereotaxic coordinates* (5th Edition). Amsterdam; Boston: Elsevier Academic Press.

Peever, J., & Fuller, P. M. (2017). The Biology of REM Sleep: Review. *Current Biology*, **27**(**22**), R1237–R1248. doi:10.1016/j.cub.2017.10.026

Perrone, S., Bellieni, C. V., Negro, S., Longini, M., Santacroce, A., Tataranno, M. L., … Buonocore, G. (2017). Oxidative Stress as a Physiological Pain Response in Full-Term Newborns. *Oxidative Medicine and Cellular Longevity*, **2017**. doi:10.1155/2017/3759287

Perry, G. S., Patil, S. P., & Presley-cantrell, L. R. (2013). Raising Awareness of Sleep as a Healthy Behavior. *Preventing Chronic Disease*, **10**(**4**), 10–13.

Peschanski, M., Guilbaud, G., Gautran, M., & Besson, J.-M. (1980). Encoding of noxious heat messages in neurons of the ventrobasal thalamic complex of the rat. *Brain Research*, **197**, 401–413. doi:10.1016/0006-8993(80)91125-7

Petrenko, A. B., Yamakura, T., Shimoji, K., & Baba, H. (2003). The Role of N-Methyl-D-Aspartate (NMDA) Receptors in Pain: A Review. *International Anesthesia Research Society*, **97**, 1108–1116. doi:10.1213/01.ANE.0000081061.12235.55

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., … Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, **2017**, 1–13. doi:10.1155/2017/8416763

Ramanathan, L., Gulyani, S., Nienhuis, R., & Siegel, J. M. (2002). Sleep deprivation decreases superoxide dismutase activity in rat hippocampus and brainstem. *Neuroreport*, **13**(**11**), 11–14.

Ramanathan, L., Hu, S., Frautschy, S. A., & Siegel, J. M. (2010). Short-term total sleep deprivation in the rat increases antioxidant responses in multiple brain regions without impairing spontaneous alternation behavior. *Behavioural Brain Research*, **207**(**2**), 305–309. doi:10.1016/j.bbr.2009.10.014

Reddi, D., Curran, N., & Stephens, R. (2013). An introduction to pain pathways and mechanisms. *British Journal of Hospital Medicine*, **74**(**12**), 188–191.

Reena, C., Ayushi, J., Pooja, S., Chhavi, S., Neha, J., & Maheep, B. (2015). Sleep deprivation: neural regulations and consequences. *Sleep and Biological Rhythms*, **13**(**3**), 210-218. doi:10.1111/sbr.12110

Roehrs, T. A., & Roth, T. (2005). Sleep and Pain : Interaction of Two Vital Functions. *Seminars in Neurology*, **25**(**1**), 106-116 doi:10.1055/s-2005-867079

Reyes, R. C., Brennan, A. M., Shen, Y., Baldwin, Y., & Swanson, R. A. (2012). Activation of neuronal NMDA receptors induces superoxide - mediated oxidative stress in neighboring neurons and astrocytes. *Journal of Neuroscience*, **32**(**37**), 12973–12978. doi:10.1523/JNEUROSCI.1597-12.2012

Rezvani, A. H. (2006). Involvement of the NMDA System in Learning and Memory Transgenic And Mutant Mice. In E. D. Levin & J. J. Buccafusco (Eds.), *Animals Models of Cognitive Impairment*. Boca Raton (FL): CRC Press/Taylor & Francis. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK2532/#_NBK2532_pubdet_

Rostami, Z., Ghasemi, S., Farzadmanesh, H., Safari, M., & Ghanbari, A. (2020). Sex Difference in Trigeminal Neuropathic Pain Response to Exercise: Role of Oxidative Stress. *Pain Research and Management*, **2020**. doi:10.1155/2020/3939757

Ruslee, S. S., Mohamad Zaid, S. S., Bakrin, I. H., Goh, Y. M., & Mohamed Mustapha, N. (2020). Protective effect of tualang honey against cadmium-induced morphological abnormalities and oxidative stress in the ovary of rats. *BMC*

Complementary Medicine and Therapies, **20**(**1**). doi:10.1186/s12906-020-02960-1

Salim, S. (2017). Oxidative stress and the central nervous system. *Journal of Pharmacology and Experimental Therapeutics*, **360**(**1**), 201–205. doi:10.1124/jpet.116.237503

Santiago, K. H., López –López, A. L., Sánchez-Muñoz, F., Cortés Altamirano, J. L., Alfaro-Rodríguez, A., & Bonilla-Jaime, H. (2021). Sleep deprivation induces oxidative stress in the liver and pancreas in young and aging rats. *Heliyon*, **7**(**3**). doi:10.1016/j.heliyon.2021.e06466

Sawamura, S., Fujinaga, M., Kingery, W. S., Belanger, N., Davies, M. F., & Maze, M. (1999). Opioidergic and adrenergic modulation of formalin-evoked spinal c-fos mRNA expression and nocifensive behavior in the rat. *European Journal of Pharmacology*, **379**(**2-3**), 141–149. doi:10.1016/S0014-2999(99)00463-X

Scammell, T. E., Arrigoni, E., & Lipton, J. O. (2017). Neural Circuitry of Wakefulness and Sleep: A Review. *Neuron*, **93**(**4**), 747–765. doi:10.1016/j.neuron.2017.01.014

Schrimpf, M., Liegl, G., Boeckle, M., Leitner, A., Geisler, P., & Pieh, C. (2015). The effect of sleep deprivation on pain perception in healthy subjects : A meta-analysis. *Sleep Medicine*, **16**(**11**). doi:10.1016/j.sleep.2015.07.022

Schuliga, M. (2015). The Inflammatory Actions of Coagulant and Fibrinolytic Proteases in Disease. *Mediators of Inflammation*, **2015**, 1–19. doi:10.1155/2015/437695

Selvaraj, P., Shanmuganathan, A., Selvaraj, S., Mani, B., Govindraj, S., & Rajan, R. (2018). Rem sleep deprivation-induced oxidative stress and its attenuation by Tephrosia purpurea (L.,) in discrete regions of rat brain. *Asian Journal of Pharmaceutical and Clinical Research*, **11**(**5**), 308–312. doi:10.22159/ajpcr.2018.v11i5.24601

Shao, Y., Lei, Y., Wang, L., Zhai, T., Jin, X., Ni, W., & Yang, Y. (2014). Altered Resting-State Amygdala Functional Connectivity after 36 Hours of Total Sleep Deprivation. *PLoS ONE*, **9**(**11**). doi:10.1371/journal.pone.0112222

Sharma, R. S., & Das, G. (2018). What is the Minimum Knowledge of Pain Medicine needed for Other Specialty ? *Journal on Recent Advances in Pain*, **4**(**1**), 32–35. doi:10.5005/jp-journals-10046-0098

Sheridan, N., & Tadi, P. (2020). Neuroanatomy, Thalamic Nuclei. In *StatPearls (Internet)* (pp. 1–5). Treasure Island (FL): StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK549908/

Shepard, J. W., Buysse, D. J., Chesson, A. L., Dement, W. C., Goldberg, R., Guilleminault, C., … White, D. P. (2005). History of the development of sleep medicine in the United States. *Journal of Clinical Sleep Medicine*, **1**(**1**), 61–82. doi:10.5664/jcsm.26298

Siegel, J. M. (2005). Functional Implications of Sleep Development. *PLoS Biology*, **3**(**5**), 756–758. doi:10.1371/journal.pbio.0030178

Silva, R. H., Abilio, V. C., Takatsu, A. L., Kameda, S. R., Grassl, C., Chehin, A. B., … Machado, R. B. (2004). Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology*, **46**, 895–903. doi:10.1016/j.neuropharm.2003.11.032

Singh, R., Kiloung, J., Singh, S., & Sharma, D. (2008). Effect of paradoxical sleep deprivation on oxidative stress parameter in brain regions of adult and old rats Effect of paradoxical sleep deprivation on oxidative stress parameters in brain regions of adult and old rats. *Biogerontology*, **9**, 153–162. doi:10.1007/s10522-008-9124-z

Siran, R., Ahmad, A. H., & Che Badariah, A. A. (2014). REM sleep deprivation induces changes of Down Regulatory Antagonist Modulator (DREAM) expression in the ventrobasal thalamic nuclei of Sprague–Dawley rats. *Journal of Physiology Biochemistry*, **70**, 877–889. doi:10.1007/s13105-014-0356-x

Šišková, Z., Justus, D., Kaneko, H., Friedrichs, D., Henneberg, N., Beutel, T., … Remy, S. (2014). Dendritic structural degeneration is functionally linked to cellular hyperexcitability in a mouse model of alzheimer's disease. *Neuron*, **84**(**5**), 1023–1033. doi:10.1016/j.neuron.2014.10.024

Slater, L., Asmerom, Y., Boskovic, D. S., Bahjri, K., Plank, Me. S., Angeles, K. R., … Angeles, D. M. (2012). Procedural Pain and Oxidative Stress in Premature Neonates. *Jornal of Pain*, **13**(**6**), 590–597. doi:10.1016/j.jpain.2012.03.010.

Solepure, A. B. (2020). Physiology of Pain: A Review. *Journal of Medical Science and Clinical Research*, **8**(**12**), 224–233.

Somanath, S., Sumiyoshi, A., Kumaran, S., Sharma, B., & Mallick, H. (2021). Thalamic Grey Matter Volume Changes After Sleep Deprivation in Rats. *Sleep and Vigilance*, **5**(**5**). doi:10.1007/s41782-021-00148-2

Somarajan, B. I., Khanday, M. A., & Mallick, B. N. (2016). Rapid eye movement sleep deprivation induces neuronal apoptosis by noradrenaline acting on alpha1 adrenoceptor and by triggering mitochondrial intrinsic pathway. *Frontiers in Neurology*, **7**, 1–14. doi:10.3389/fneur.2016.00025

Spencer, J. P. E. (2008). Food for thought: The role of dietary flavonoids in enhancing human memory, learning and neuro-cognitive performance. *Proceedings of the Nutrition Society*, **67**(**2**), 238–252. doi:10.1017/S0029665108007088

Steeds, C. E. (2016). The anatomy and physiology of pain. *Surgery*, **34**(**2**), 55–59. doi:10.1016/j.mpsur.2015.11.005

Steriade, M. (2005). Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends in Neurosciences*, **28**(**6),** 317–324. doi:10.1016/j.tins.2005.03.007

Squire, L. R., Bloom, F. E., McConnell, S. K., Roberts, J. L., Spitzer, N. C., & Zigmond, M. J. (2003). *Fundamental Neuroscience* (2nd editio). Academic Press.

Suchecki, D., Tiba, P. A., & Machado, R. B. (2012). REM sleep rebound as an adaptive response to stressful situations. *Neurology*, **3**, 1–12. doi:10.3389/fneur.2012.00041

Sufka, K. J., & Price, D. D. (2002). Gate Control Theory Reconsidered. *Brain and Mind*, **3**, 277–290. doi:10.1023/A

Swenson, R. S. (2006). The cerebral cortex. In *Review of clinical and functional neuroscience*. United State: Dartmouth Medical School.

Tang, S. P., Sirajudeen, K. N. S., Jaafar, H., Gan, S. H., Muzaimi, M., & Sulaiman, S. A. (2017). Tualang Honey Protects the Rat Midbrain and Lung against Repeated Paraquat Exposure. *Oxidative Medicine and Cellular Longevity*. doi:10.1155/2017/4605782

Tao, Z., Wang, P., Wei, S., Traub, R. J., Li, J., & Cao, D. (2019). Review Article The Role of Descending Pain Modulation in Chronic Primary Pain: Potential Application of Drugs Targeting Serotonergic System. *Neural Plasticity*, **2019**, 1–16.

Tengku Adnan, T. F., Amin, A., Abd Aziz, C. B., Wan Ahmad, W. A. N., & Noordin, L. (2017). REM Sleep Deprivation is Associated with Morphological and Functional Changes of Vascular Endothelium REM Sleep Deprivation is Associated with Morphological and Functional Changes of Vascular Endothelium. *Annals of Microscopy*, **16**, 4-12.

Terao, A., Greco, M. A., & Genome, S. (2003). Changes In Immediate Expression In Response To Sleep Deprivation And Recovery Sleep In The Mouse Brain. *Neuroscience*, **120**, 1115–1124. doi:10.1016/S0306-4522(03)00395-6

Thamaraiselvi, K., Mathangi, D. C., & Subhashini, A. S. (2012). Effect of increase in duration of REM sleep deprivation on lipid peroxidation. *International Journal of Biology & Medical Research*, **3**(**2**), 1754–1759.

Torabi-Nami, M., Nasehi, M., & Zarrindast, M. R. (2013). Sleep loss and the brain vulnerability to neurodegeneration: Behavioral, biochemical and neuro-histopathological observations in a rat model. *EXCLI Journal*, **12**(June 2014), 347–372. doi:10.17877/DE290R-5603

Torrico, T. J., & Munakomi, S. (2020). Neuroanatomy, thalamus. In *StatPearls (Internet)*. Treasure Island (FL): StatPearls Publishing.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry and Cell Biology*, **39**(**1**), 44–84. doi:10.1016/j.biocel.2006.07.001

Vanini, G. (2016). Sleep deprivation and recovery sleep prior to a noxious inflammatory insult influence characteristics and duration of pain. *Sleep*, **39**(**1**), 133–142. doi:10.5665/sleep.5334

Vaziri, D. N., Wang, X. Q., Oveisi, F., & Rad, B. (2000). Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension*, **36**(**1**), 142–146. doi:10.1161/01.HYP.36.1.142

Villafuerte, G., Miguel-puga, A., Rodríguez, E. M., Machado, S., Manjarrez, E., & Arias-carrión, O. (2015). Sleep Deprivation and Oxidative Stress in Animal Models : A Systematic Review. *Oxidative Medicine and Cellular Longetivity*, **2015**.

Wan Ghazali, W. S., Mohamed, M., Sulaiman, S. A., Aziz, A. A., & Yusoff, H. M. (2015). Tualang honey supplementation improves oxidative stress status among chronic smokers. *Toxicological and Environmental Chemistry*, **97**(**8**), 1017–1024. doi:10.1080/02772248.2015.1077959

Wan Yusuf, W. N., Wan Mohammad, W. M. Z., Siew, H. G., Mustafa, M., Abd Aziz, C. B., & Sulaiman, S. A. (2019). Journal of Traditional and Complementary Medicine Tualang honey ameliorates viral load, CD4 counts and improves quality of life in asymptomatic human immunode fi ciency virus infected patients. *Journal of Traditional Chinese Medical Sciences*, **9**(**4**), 249–256. doi:10.1016/j.jtcme.2018.05.003

Watkins, J. C., & Jane, D. E. (2006). The glutamate story. *Journal of Pharmacology*, **147**, 100–108. doi:10.1038/sj.bjp.0706444

Wei, S., Tao, Z., Xue, Y., & Cao, D. (2020). Peripheral Sensitization. In H. Turker, L. Garcia Benavides, G. Ramos Gallardo, & M. MÃ©ndez Del Villar (Eds.), *Peripheral Nerve Disorder and Treatment*. London, UK: InTechOpen. doi:http://dx.doi.org/10.5772/intechopen.90319 4.

Woolf, C. J., & Thompson, S. W. N. (1991). The induction and maintenance of central sensitization is dependent in N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain*, **44**, 293–299. doi:10.1016/0304-3959(91)90100-C

Yam, M. F., Loh, Y. C., Tan, C. S., Adam, S. K., Abdul Manan, N., & Basir, R. (2018). General Pathways of Pain Sensation and the Major Neurotransmitters Involved in Pain Regulation. *International Journal of Molecular Sciences*, **19**(**2164**). doi:10.3390/ijms19082164

Yang, M. S., Chan, H. W., & Yu, L. C. (2006). Glutathione peroxidase and glutathione reductase activities are partially responsible for determining the susceptibility of cells to oxidative stress. *Toxicology*, 226(2–3), 126–130. doi:10.1016/j.tox.2006.06.008

Yen, C., & Lu, P. (2013). Thalamus and pain. *Acta Anaesthesiologica Taiwanica*, **51**(**2**), 73–80. doi:10.1016/j.aat.2013.06.011

Yowtak, J., Lee, K. Y., Kim, H. Y., Wang, J., Kim, H. K., Chung, K., & Chung, J. M. (2011). Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. *Pain*, **152**(**4**), 844–852. doi:10.1016/j.pain.2010.12.034

Zaidi, S. M. K. R., Al-Qirim, T. M., Hoda, N., & Banu, N. (2003). Modulation of restraint stress induced oxidative changes in rats by antioxidant vitamins. *Journal of Nutritional Biochemistry*, **14**(**11**), 633–636. doi:10.1016/S0955-2863(03)00117-7

Zelman, D. C., Brandenburg, N. A., & Gore, M. (2006). Sleep Impairment in Patients With Painful Diabetic Peripheral Neuropathy. *The Clinical Journal of Pain*, **22**(**8**), 681–685.

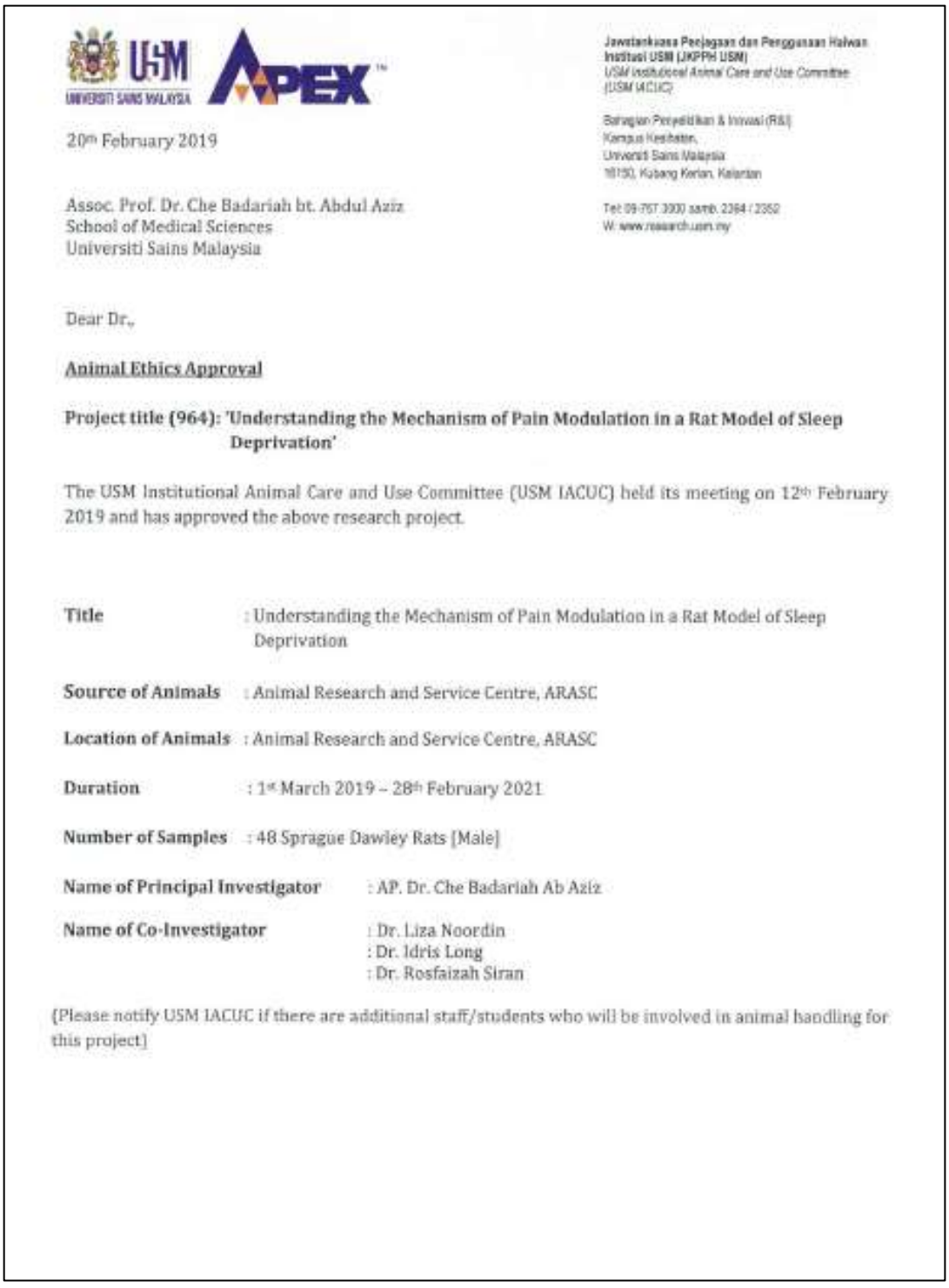
Zhang, H. L., Pan, F., Hong, D., Shenoy, S. M., Singer, R. H., & Bassell, G. J. (2003). Active transport of the survival motor neuron protein and the role of exon-7 in cytoplasmic localization. *Journal of Neuroscience*, **23**(**16**), 6627–6637. doi:10.1523/jneurosci.23-16-06627.2003

Zhang, L., Guo, H. L., Zhang, H. Q., Xu, T. Q., He, B., Wang, Z. H., … Liu, F. E. (2017). Melatonin prevents sleep deprivation-associated anxiety-like behavior in rats: Role of oxidative stress and balance between gabaergic and glutamatergic transmission. *American Journal of Translational Research*, **9**(**5**), 2231–2242.

Zhou, Q., & Sheng, M. (2013). Neuropharmacology Invited review NMDA receptors in nervous system diseases. *Neuropharmacology*, **74**, 69–75. doi:10.1016/j.neuropharm.2013.03.030

APPENDICES

Appendix A: Letter of Approval from Animal Ethics Committee USM



Jawatankuasa Penjagaan dan Penggunaan Haiwan
Institusi USM (JKPPH USM)
USM Institutional Animal Care and Use Committee
(USM IACUC)

Bahagian Penyelidikan & Inovasi (R&I)
Kampus Kesihatan,
Universiti Sains Malaysia
16150, Kubang Kerian, Kelantan

Tel: 09-767 3000 samb. 2264 / 2352
W: www.research.usm.my

20th February 2019

Assoc. Prof. Dr. Che Badariah bt. Abdul Aziz
School of Medical Sciences
Universiti Sains Malaysia

Dear Dr.,

Animal Ethics Approval

Project title (964): 'Understanding the Mechanism of Pain Modulation in a Rat Model of Sleep Deprivation'

The USM Institutional Animal Care and Use Committee (USM IACUC) held its meeting on 12th February 2019 and has approved the above research project.

Title : Understanding the Mechanism of Pain Modulation in a Rat Model of Sleep Deprivation

Source of Animals : Animal Research and Service Centre, ARASC

Location of Animals : Animal Research and Service Centre, ARASC

Duration : 1st March 2019 – 28th February 2021

Number of Samples : 48 Sprague Dawley Rats [Male]

Name of Principal Investigator : AP. Dr. Che Badariah Ab Aziz

Name of Co-Investigator : Dr. Liza Noordin
: Dr. Idris Long
: Dr. Rosfaizah Siran

(Please notify USM IACUC if there are additional staff/students who will be involved in animal handling for this project)

The following items (X) were received and reviewed in connection with the above study to be conducted by the investigator.

(X) Copy of Proposal	(Date : 18th December 2018)
(X) Animal Ethics Committee Approval Application Form	(Date : 18th December 2018)
(X) Reviewer's Comment Form	(Date : 13th January 2019)
(X) Reply for Clarification Letter	(Date : 18th February 2019)

Members of the Animal Ethics Committee USM who reviewed the documents as follows:

Member (Title and Name)		Occupation (Designation)
Chairperson: Prof. Dr. Aida Hanum Ghulam Rasool		Lecturer, School of Medical Sciences (Pharmacology)
Secretary: Siti Amirah Nasir		Secretary
Members:		
1	Assoc. Prof. Dr. Mohd Fadhli Khamis	Dean, School of Dental Sciences (Forensic Dentistry/Statistician)
2	Assoc. Prof. Dr. Rapeah Suppian	Lecturer, School of Health Sciences (Immunology)
3	Assoc. Prof. Dr. Mahaneem Mohamed	Lecturer, School of Medical Sciences (Physiology)
4	Assoc. Prof. Dr. Lim Boon Huat	Lecturer, School of Health Sciences (Medical Parasitology/Molecular Diagnostic)
5	Dr. Nor Aini Saidin	Lecturer, Advanced Medical & Dental Institute (Toxicology)
6	Dr. Saidi Jaafar	Lecturer, School of Dental Sciences (Developmental Biology/Knock-out & Transgenic)
7	Dr. Basariuddin Ahmad	Lecturer, School of Dental Sciences (Statistician)
8	Dr. Isma Suzyta Ismail	Veterinary Officer, ARASC
9	Dr. Noziah Ghani	Veterinary Officer, ARASC
10	Pn. Hjh. Seri Dewi Ibrahim	Lavperson

Thank you.

"Ensuring a Sustainable Tomorrow"

PROF. DR. AIDA HANUM GHULAM RASOOL
Chairperson
USM Institutional Animal Care and Use Committee (USM IACUC)

C c : Animal Research and Service Centre, ARASC

Appendix B: Formalin Test Score Form

Group:
Bil:
Date:

Duration	Min 1	Min 2	Min 3	Min 4	Min 5	MEAN
0-5 min						
	Min 6	Min 7	Min 8	Min 9	Min 10	MEAN
6-10 min						
	Min 11	Min 12	Min 13	Min 14	Min 15	MEAN
11-15 min						
	Min 16	Min 17	Min 18	Min 19	Min 20	MEAN
16-20 min						
	Min 21	Min 22	Min 23	Min 24	Min 25	MEAN
21-25 min						
	Min 26	Min 27	Min 28	Min 29	Min 30	MEAN
26-30 min						
	Min 31	Min 32	Min 33	Min 34	Min 35	MEAN
31-35 min						
	Min 36	Min 37	Min 38	Min 39	Min 40	MEAN
36-40 min						
	Min 41	Min 42	Min 43	Min 44	Min 45	MEAN
41-45 min						
	Min 46	Min 47	Min 48	Min 49	Min 50	MEAN
46-50 min						
	Min 51	Min 52	Min 53	Min 54	Min 55	MEAN
51-55 min						
	Min 56	Min 57	Min 58	Min 59	Min 60	MEAN
56-60 min						

LISTS OF PUBLICATIONS AND PRESENTATIONS

1. Publications

    1.1 Mohd Shafie, A. S., Abd aziz, C. B., Noordin, L., Long, I., Mat Zin, A. A., & Siran, R. (2021). Tualang honey modulated nociceptive responses in the thalamus of REM sleep deprivation rat model. *Malaysian Applied Biology*, **50**(**2**), 1–9.

    1.2 Mohd Shafie, A. S., Long, I., Noordin, L., & Abd Aziz, C. B. (2021). Modulation of pain behavior responses by Tualang honey in a rat model of sleep deprivation. *Journal of Complementary and Integrative Medicine*, **18**(**4**), S1-S57.

    1.3 Mohd Shafie, A. S., Abd aziz, C. B., Long, I., Siran, R., & Noordin, L. (2021). Impact of Rapid Eye Movement (REM) sleep deprivation on pain behaviour and oxidative stress in the thalamus: Role of Tualang honey supplementation. *Malaysia Journal of Medical Sciences* (Accepted)

2. Presentations

    2.1 Title: Oxidative Stress in the Thalamus Modulated Pain Responses in REM Sleep Deprivation Rat Model

    Venue: International Postgraduate e-Symposium (IPeS) 2021, School of Health Sciences, Health Campus, Universiti Sains Malaysia, Kota Bharu.

    Date: 1st – 2nd August 2021

2.2 Title: Modulation of Pain Behaviour Responses by Tualang Honey in a Rat Model of Sleep Deprivation

Venue: 1st International Virtual Conference on Integrative Medicine 2021 (e-ICIM), Advance Medical and Dental Institute, Universiti Sains Malaysia, Penang

Date: 17th -19th August 2021