THE ELECTROPHYSIOLOGICAL AND BEHAVIOURAL EFFECTS OF SUBCHRONIC ADMINISTRATION OF STANDARDISED METHANOLIC *MITRAGYNA SPECIOSA* EXTRACT ON ADULT MALE SPRAGUE-DAWLEY RATS

by

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LIST OF ABBREVIATIONS

aCSF	Artificial Cerebrospinal Fluid
AMPA	2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid
CA	Cornu Ammonis
CMC	Carboxymethylcellulose
CS	Conditioned Stimulus
EGTA	Ethylene Glycol Tetraacetic Acid
fEPSP	Field Excitatory Postsynaptic Potential
GC-MS	Gas Chromatography Mass Spectrometry
H&E	Haematoxyline and Eosin
HFS	High Frequency Stimulation
LTP	Long-Term Potentiation
MeOh	Methanol
NMDA	N-Methyl-D-Aspartate
NMDAR	N-Methyl-D-Aspartate Receptor
PA	Passive Avoidance
rpm	Revolutions per Minute
RT	Retention Time
SEM	Standard Error Mean
SMMSE	Standardised Methanolic *Mitragyna Speciosa* Extract
SPSS	Statistical Package for the Social Sciences
STL	Step-through Latency
STP	Short-term Potentiation
US	Unconditioned Stimulus

KESAN ELEKTROFISIOLOGI DAN TINGKAH LAKU ADMINISTRASI SUBKRONIK EKSTRAK METANOLIK *MITRAGYNA SPECIOSA* TERPIAWAI KE ATAS TIKUS SPRAGUE-DAWLEY JANTAN DEWASA

ABSTRAK

Kajian ke atas *Mitragyna speciosa* Korth atau dikenali sebagai ketum telah dijalankan bagi menentukan kesannya terhadap pembelajaran dan ingatan serta kesan toksiknya terhadap haiwan yang terdedah secara subkronik. Terdapat tiga objektif yang telah dijalankan bagi melengkapkan kajian ini. Objektif pertama adalah untuk menentukan ciri-ciri elektrofisiologi ekstrak metanolik *Mitragyna speciosa* terpiawai (EMMST) terhadap hirisan hipokampus yang didedahkan secara subakut (14 hari) dan subkronik (28 hari) melalui kesannya terhadap potensiasi jangka panjang (PJP). Objektif yang kedua adalah untuk mengkaji kesan pendedahan subkronik EMMST terhadap fungsi kognitif dengan menggunakan ujian pengelakan pasif. Objektif yang terakhir adalah untuk mengkaji kesan toksisiti pendedahan subkronik EMMST ke atas histopatologi organ, hematologi dan analisis biokimia. Bagi objektif yang pertama, haiwan dibahagikan kepada 4 kumpulan; kawalan, MS100 (100 mg/kg EMMST), MS200 (200 mg/kg EMMST) dan MS500 (500 mg/kg EMMST). Rakaman PJP untuk haiwan yang didedahkan kepada EMMST selama 14 hari menunjukkan bahawa aruhan PJP dihalang sebahagian. Manakala, pendedahan selama 28 hari menunjukkan aruhan PJP dihalang sepenuhnya. Bagi objektif kedua, haiwan dibahagikan kepada 5 kumpulan, iaitu kawalan, MS100, MS200, MS 500 dan tambahan kumpulan morfina (10 mg/kg). Di dalam ujian pengelakan pasif, semua kumpulan menunjukkan pembelajaran pada fasa

perolehan. Manakala, pada fasa retensi, haiwan yang didedahkan kepada EMMST secara subkronik menunjukkan peningkatan ingatan, terutamanya pada kumpulan MS500. Untuk objektif yang terakhir, EMMST bagi ke semua dos adalah positif toksik terhadap badan. Melalui histopatologi, organ yang didapati terkesan dengan ketoksikan pendedahan subkronik EMMST adalah hati, ginjal dan paru-paru. Kesan toksik juga kelihatan pada analisa biokimia terutamanya pada AST (aspartat aminotransferase), kreatinina, globulin, glukosa, jumlah protein dan urea. Selain itu, tiada sebarang perbezaan yang signifikan bagi ujian hematologi. Kesimpulannya, pendedahan subkronik ketum mampu meningkatkan pembelajaran dan ingatan. Walaubagaimanapun, mekanisme utama pembelajaran dan ingatan iaitu aruhan LTP dihalang sepenuhnya oleh ekstrak tersebut. Pendedahan subkronik ekstrak tersebut juga adalah toksik kepada hati, peparu dan ginjal.

THE ELECTROPHYSIOLOGICAL AND BEHAVIOURAL EFFECTS OF SUBCHRONIC ADMINISTRATION OF STANDARDISED METHANOLIC *MITRAGYNA SPECIOSA* EXTRACT ON ADULT MALE SPRAGUE-DAWLEY RATS

ABSTRACT

The research on *Mitragyna speciosa* Korth or known as ketum has been carried out to determine its effects towards learning and memory as well as its toxic effects on animals that have been exposed subchronically. There were three objectives in the present study. The first objective was to study the electrophysiological characteristics of standardised methanolic *Mitragyna speciosa* extract (SMMSE) on hippocampal slices of rats that were exposed to subacute (14 days) and subchronic (28 days) treatment via its effect on long-term potentiation (LTP). The second objective was to study the effects of subchronic SMMSE towards cognitive function by using passive avoidance (PA) test. The last objective was to study the toxicity effects of subchronic SMMSE exposure on organ histopathology, haematology and biochemical parameters. For the first objective, animals were divided into four groups; control, MS100 (100 mg/kg SMMSE), MS200 (200 mg/kg SMMSE) and MS500 (500 mg/kg SMMSE). LTP recording of animals that were exposed to SMMSE for 14 days showed that LTP induction was partially blocked. Meanwhile, the exposure for 28 days showed that LTP induction was totally blocked. For the second objective, animals were divided into five groups; control, MS100, MS200, MS500 and additional morphine group (10 mg/kg). In the passive avoidance test, all groups learned in the acquisition phase. Meanwhile, in the retention phase,

subchronically exposed groups showed the improvement in memory, especially MS500. For the last objective, all doses of SMMSE were potentially toxic to the body. Histopathology analyses showed that organs affected by the toxicity of subchronic SMMSE were liver, kidney and lungs. Toxic effects were also seen in the biochemical analyses especially on AST (aspartate aminotransferase), creatinine, globulin, glucose, total protein as well as urea. In contrast, there were no significant differences for hematology tests. In conclusion, subchronic exposure of ketum was able to improve learning and memory. However, the main mechanism of learning and memory which was LTP was totally inhibited by the extract. The subchronic exposure of the extract was also toxic to the liver, lung and kidney.

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Knowledge is gained through learning process and sustained through memory. Memory is defined as a behavioural change caused by an experience and learning refers to the process for acquiring memory. Memory can be divided into short-term and long-term memory (Blum *et al*., 2009). Short-term memory takes place in prefrontal cortex while long-term memory takes place in the hippocampus and the amygdala. Synaptic plasticity is one of the important neurochemical foundations of learning and memory (Deng *et al*., 2010).

Synaptic plasticity is defined as an increase in synaptic efficacy that arises from the presynaptic cell's repeated and persistent stimulation of the postsynaptic cell (Hebb, 1949). The theory that supports synaptic plasticity which is known as Hebbian Rule states that the cellular basis of learning involves strengthening of a synapse that is repeatedly activated when the postsynaptic neuron fires. One of the underlying mechanisms of synaptic plasticity is long-term potentiation (LTP). LTP is simply defined as long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously (Cooke & Bliss, 2006; Ohno, 2011).

Briefly, LTP is induced by an activation of glutamatergic system between synaptic connections amongst neurons. There are specific receptors involved in this system such as AMPA and NMDA receptors. These receptors will be activated upon the

binding of specific neurotransmitters and allow certain ions e.g. Ca^{2+}, to pass through the cell membrane. Hence, this allows the propagation of action potential for the entire neuronal connections. Therefore, through the understanding of how the system works, this research was partly conducted to seek the effects on LTP upon the introduction of foreign compounds to the system.

Ketum or *Mitragyna speciosa* Korth is an indigenous plant mainly found in Southeast Asia regions. This plant belongs to the Rubiaceae family which is also a family of the coffee tree. In addition of being used as a narcotic drug, it is often used as a substitute for opium. Ketum has also been reported to be a central nervous system stimulant rather than a depressant. Extraction of alkaloids by previous studies showed that ketum consists of over 20 alkaloids (Kapp *et al*., 2011). Four main alkaloids are mitragynine, speciogynine, paynantheine with small amounts of speciociliatine. However, in this study, crude extract was used instead of the alkaloid extract. The reason for this was to mimic the real human usage of ketum in everyday life which is mostly by chewing or drinking the water decoction of the leaves. Matsumoto and colleagues (2005) reported that mitragynine blocked KCl-induced Ca^{2+} influx in neuronal cells while rhynchophylline which consists of about 10% in the total alkaloids reduced glutamate-induced Ca^{2+} influx and protected against glutamate-induced neuronal death in cultured cerebellar granule cells (Shimada *et al*., 1999).

Information obtained from the understanding of the effects of ketum on brain is useful to study its effects on learning and memory which can be narrowed down to LTP and glutamatergic system. This research applied subchronic administration of

standardised methanolic *Mitragyna speciosa* extract (SMMSE) on the LTP induction and the cognitive function.

1.2 *Mitragyna speciosa* Korth

The botanical name *Mitragyna speciosa* Korth or also known as ketum in Malaysia, belongs to Rubiaeceae (Idid *et al*., 1998), a coffee family. It is also called biak-biak, kratom, kakuam, ithang and thom. Ketum is a large tree found ubiquitously in Southeast Asia region especially Malaysia and Thailand. Ketum is used traditionally in folk medicine although it is claimed to cause addiction (Chittrakarn *et al.,* 2008). According to Kumarnsit and colleagues (2006), the leaves are the most effective part. It is used as analgesic, antipyretic, antidiarrheal, and local anesthetic.

Figure 1.1 *Mitragyna speciosa* plant or ketum is largely found in North Malaysia and Thailand.

1.2.1 Studies of the Effects of *Mitragyna Speciosa* Korth Standardised Methanolic Extract

The effects of *Mitragyna speciosa* Korth standardised methanolic extract in Malaysia have been detected in several acute studies conducted in the Department of Neurosciences, Universiti Sains Malaysia. Harizal and colleagues (2010) reported the acute toxicity effects of *Mitragyna speciosa* Korth standardised methanolic extract in rodents. Generally, they found that there were significant elevation of AST, ALT, albumin, triglycerides, cholesterol and creatinine. Furthermore, histological examination showed congestion of sinusoids, hemorrhage hepatocytes, fatty change, centrilobular necrosis and increased number of Kuppfer cells in liver of all treated groups.

In another study, Senik *et al*, (2012a) reported that the acute administration of *Mitragyna speciosa* Korth standardised methanolic extract facilitated learning, but there was no benefit on long term memory consolidation. In the same year, Senik and friends reported the effects of the same extract on the long-term potentiation (LTP) induction of CA1 subfield in rat hippocampal slices. The concentration of the extract at 0.008% prevented the induction of LTP and induced only short-term potentiation in CA1 neurons.

Thus, as the continuity from the previous studies the present study was conducted by extending the period of administration to subchronic with slight modification. The reason for that was to provide immense details on the effects of *Mitragyna speciosa* Korth standardised methanolic extract.

1.2.2 Other Effects of Ketum

Originally, local people used ketum to alleviate pain, coughing or diarrhoea. It is also used to prevent fatigue (Suwanlert, 1975). The leaf has been used in Thailand for its opium-like effect (Burkill, 1935). In addition to being used in its own right, it is often used to replace opium when opium is not available. Some drug users are trying to moderate their opium addiction by taking ketum instead. Ketum has been reported to be a central nervous system stimulant rather than a depressant (Suwanlert, 1975). Nowadays, it has gained so much attention since there are claims that it can inhibit withdrawal symptoms that follow cessation from long-term ethanol consumption and prevent ethanol patients from relapsing (Kumarnsit *et al*., 2007). The study by Kumarnsit and colleagues (2007) on the effect of ketum concluded that aqueous extract of ketum was able to inhibit withdrawal behaviours in mice including rearing, displacement and head weaving. Results by Chittrakarn showed that methanolic ketum extract exhibited its antidiarrheal effect on rat gastrointestinal tract (Chittrakarn *et al.*, 2008). Acute administration of alkaloid extract of ketum significantly resulted in dose-dependent decreases in food and water intake in rats. Furthermore, prolonged suppressing effects were observed following administration of the ketum extract for 60 days and also significantly suppressed weight gaining (Kumarnsit *et al*., 2006). Standardised methanolic extract of ketum has been reported to increase blood pressure after an hour of administration. High dose of the extract also induce acute severe hepatotoxicity and mild nephrotoxicity. However, the extract has no effect on body weight, food and water consumption, absolute and relative organ weight and haematology test (Harizal *et al*., 2010). The presence of antinociceptive effect in various extracts (alkaloid, methanolic and aqueous) of Malaysian ketum leaves has been

reported. Furthermore, antinociceptive doses vary according to the type of solvents used for extraction (Sabetghadam *et al*., 2010).

1.2.3 The Alkaloids of Ketum

There are altogether over 20 alkaloids that have been isolated from the leaves of ketum. However, the content varies from location to location and from time to time. The main indole alkaloidal content is fairly stable and it would appear that mitragynine, speciogynine, paynantheine with small amounts of speciociliatine are present in all leaves (Shellard, 1974). Rhynchophylline has also been reported to be one of the constituents in that species (Trager, 1968; Hassan *et al*., 2013). Mitragynine ($C_{23}H_{30}N_2O_4$, exact molecular mass = 398.2207) is the major alkaloid. Mitragynine has been reported to have similar effect with that of codeine as an analgesic in dogs, but it was considered to act at opioid receptors (Macko *et al*., 1972; Jansen and Prast, 1988). Additionally, a study reported that mitragynine has an opioid receptor agonist effect on guinea-pig ileum contraction (Watanabe *et al.,* 1992, Watanabe *et al.,* 1997). Mitragynine also has an antinociceptive action through the supraspinal opioid receptors, and that its action is dominantly mediated by μ- and δ-receptor subtypes in *in vivo* and *in vitro* studies (Matsumoto *et al*., 1996; Tohda *et al*., 1997; Thongpradichote *et al.,* 1998). A study by Yamamoto and colleagues (1999) showed that mitragynine and its derivative compound mitragynine pseudoindoxyl as potent agonists in *in vitro* experiments. Mitragynine and mitragynine pseudoindoxyl inhibit the vagally stimulated twitch contraction through opioid receptors in guinea-pig ileum. The effect was 20-fold more potent than that of morphine. Whilst in mouse vas deferens, the effect of mitragynine pseudoindoxyl was 35-fold more potent than morphine.

Table 1.1 The profile of alkaloids found in *Mitragyna speciosa* (Hassan *et al*., 2013).

Alkaloid	Percentage (%)
Mitragynine	66
Paynantheine	9
Speciogynine	7
7-hydroxymitragynine	2
Speciociliatine	1
Mitraphylline	<1
Isomitraphylline	<1
Speciophylline	<1
Rhynchophylline	<1
Isorhyhnchophylline	<1
Ajmalicine	<1
Corynantheidine	<1
Corynoxine A	<1
Corynoxine B	<1
Mitrafoline	<1
Isomitrafoline	<1
Oxindale A	<1
Oxindole B	<1
Speciofoline	<1
Isospeciofoline	<1
Ciliaphylline	<1
Mitraciliatine	<1
Mitragynaline	<1
Mitragynalinic acid	<1
Corynantheidalinic acid	<1

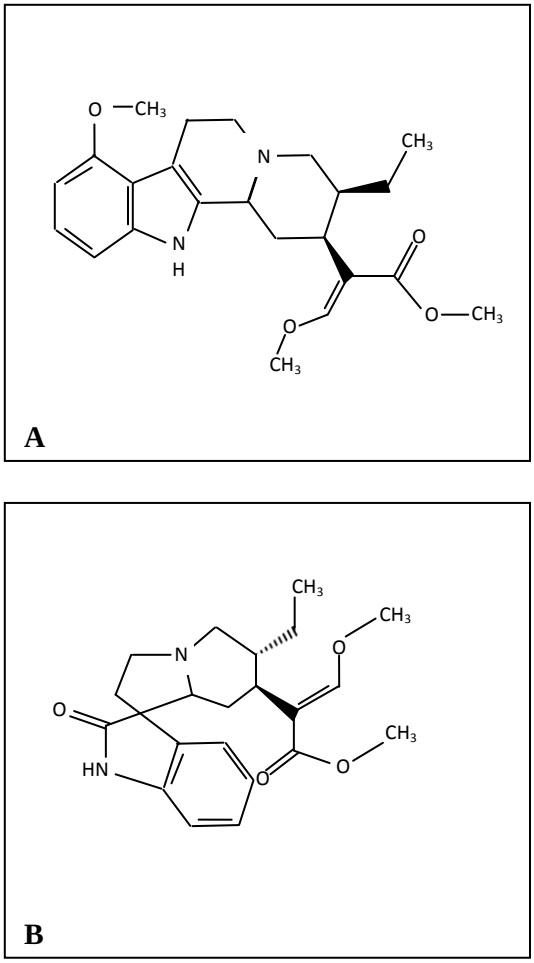

Figure 1.2 The chemical structures of two alkaloids of *Mitragyna speciosa*. **A)** Mitragynine is an indole alkaloid of *Mitragyna speciosa.* **B)** Rhynchophylline is the main alkaloid for certain *Uncaria* species

Mitragynine has also been reported to be very potent μ- and δ-opioid agonist in both functional and binding assays (Yamamoto *et al.,* 1999). Mitragynine pseudoindoxyl induced only weak antinociceptive effect in mouse tail-flick test in comparison with morphine (Takayama *et al*., 2002). By using the patch-clamp technique, mitragynine was found to block T- and L-type Ca^{2+} channel currents in N1E-115 neuroblastoma cells. In the Ca^{2+} measurement by a fluorescent dye method, mitragynine reduced KCl-induced Ca^{2+} influx in neuroblastoma cells (Matsumoto *et al*., 2005). Chronic administration of mitragynine significantly reduced locomotor activity in open field test compared to normal group. Furthermore, for object location task, mitragynine did not show significant discrimination between the object that had changed position than the object that remained in a constant position. Thus, chronic administration of mitragynine can alter the cognitive behavioural function in mice (Apryani *et al*., 2010). The study on the metabolism of mitragynine in rat and human urine using liquid chromatography-linear ion trap mass spectrometry showed that four metabolites were additionally conjugated to glucoronides and one to sulfate in rats. Meanwhile, three metabolites were conjugated to glucoronides and three to sulfates in humans. This study suggested that mitragynine was extensively metabolised in rats and humans with some differences (Philipp *et al*., 2009). Rhynchophylline, one of the alkaloids found in *Mitragyna speciosa* has been reported to reduce glutamate-induced Ca^{2+} influx and protected against glutamate-induced neuronal death in cultured cerebellar granule cells (Shimada *et al.,* 1999). In other words, rhynchophylline acts by blocking Ca^{2+} channel and opening K^{+} channel. Acute oral administration of the alkaloid extract or mitragynine significantly increased the total number of arm entries, rearing frequency and decreased grooming and immobility time in Y-maze test when compared to control group. However, the

alkaloid extract or mitragynine do not affect short-term memory or motor coordination (Hazim *et al*., 2011).

1.3 Hippocampus

The hippocampus is defined as a curved elevation in the floor of the inferior horn of the lateral ventricle. It is also a functional component of the limbic system and its efferent projections from the fornix. Hippocampus plays important roles in consolidation of information from short-term to long-term memory and spatial navigation. As human's brain is divided into two hemispheres, hippocampus occupies both hemispheres. The word hippocampus is Latin for "seahorse". The hippocampi lie on the inner side of the temporal lobes (just below the temples along the sides of the head) in an area called medial temporal lobe. Some early neuroanatomists noted that this shape bore a resemblance to the horns of a ram. In fact, another name for the hippocampus is *cornuammonis,* of the "Ammon's horn", after the Egyptian god Amon and hence named the subfields in hippocampus as CA (cornuammonis) 1 through 4. There are other parts in and nearby the hippocampus which are dentate gyrus, subiculum, entorhinal cortex, perirhinal cortex, parahippocampal cortex and amygdala that respectively functioning differently (Duvernoy, 2005).

1.3.1 The Role of Hippocampus

The hippocampus is one of the parts in the brain that form the limbic system. Different components of the limbic system play different roles in various aspects such as emotions, fear, learning and memory (Cardinal *et al*., 2002; Del Arco and Mora, 2009). A study conducted by Scoville and Milner (1957) to identify the role of hippocampus started with the study on amnesia in human patients following removal of the hippocampus and neighbouring medial temporal structures. The hippocampus and other related structures are involved in the formation of episodic memories in human as well as in consolidating memory (Mumby *et al*., 1999). Besides the crucial role in learning and memory, hippocampus has been studied to play its function in anxiety. A study by Engin and Treit (2007) reported that hippocampus is a neural mediator of emotion. Intrahippocampal infusions of glutamatergic, serotonergic and cholinergic compounds also produce statistically significant antianxiety effects. However, the results vary as a function of specific anxiety reactions and to a certain extent, specific intrahippocampal targets. There were studies conducted to prove the role of hippocampus in pathophysiology of major depressive disorder (MDD). In animal studies, the increment of glucocoticoid levels associated with MDD may negatively affect neurogenesis that will cause excitotoxic damage in hippocampus complex (Campbell and MacQueen, 2004).

1.3.2 The Anatomy of Hippocampus

The term hippocampal formation includes the dentate gyrus, hippocampus proper, subicular complex (subiculum, presubiculum and parasubiculum) and entorhinal cortex (Figure 1.3)(Van Strien *et al*., 2009). The anatomy and circuitry of these regions are largely conserved across mammalian species. The hippocampal formation is located in a very similar location in all mammals. Macroscopically, it is close to olfactory structures. Two grooves in the brain called rhinal sulcus and the hippocampal fissures are key anatomical landmarks that define to a great extent the limits of the hippocampal formation in the brain. The rhinal sulcus is the border between the neocortex and the entorhinal cortex. The smaller the size of the neocortex, the longer and more externally visible the rhinal sulcus is. In man and primates, the rhinal sulcus is largely hidden in the ventromedial aspect of the temporal lobe. The rhinal sulcus in nonhuman primates runs longitudinally for about one half of the rostro caudal surface of the ventral temporal lobe, but in man the rhinal sulcus is very short, vertically oriented and in close proximity to the medial aspect of the frontotemporal junction (limen insulae). While in other mammals, the rhinal sulcus is the border between the neocortex and the entorhinal cortex. In man, this border is situated in the medial bank and shoulder of the collateral sulcus (a long sulcus, parallel to the major axis of the human temporal lobe). Hence, it can be said that the rhinal sulcus is a constant feature of the mammalian brain where the hippocampal formation meets the neocortex (Insausti, 1993).

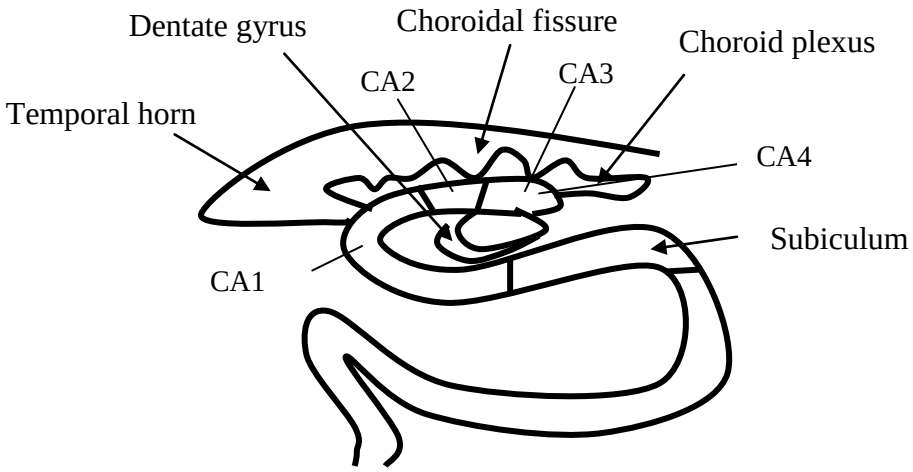

Figure 1.3 The anatomical diagram of general hippocampus. The term hippocampal formation typically refers to the dentate gyrus, the hippocampal proper and the subicular cortex.

1.3.3 The Hippocampal Network

The hippocampus has a principally unidirectional network. Input from entorhinal cortex forms a connection with the dentate gyrus and the CA3 pyramidal neurons via perforant pathway. Mossy fibre is another pathway that connects the dentate gyrus with the CA3 pyramidal neurons. The next pathway is Schaffer collateral which connects CA3 pyramidal neurons with CA1 pyramidal neurons (Figure 1.4)(Van Strien *et al*., 2009).

Perforant pathway is the major input to the hippocampus. The axons of the perforant pathway arise in certain layers of the entorhinal cortex. The axons are projected to the granule cells of dentate gyrus and pyramidal cells of CA3 region (Witter *et al*., 1988). It was in this pathway that long-term potentiation was first discovered.

The axons of the dentate gyrus granule cells elongate from dentate gyrus to CA3 region to make the mossy fibre pathway, forming a major input. Mossy fibre synapses in CA neurons consist quite a number of termini, with a large amount of neurotransmitter release sites and post-synaptic densities. The pathway is extensively studied for kainate functions in synaptic plasticity (DeltaRS, 2012).

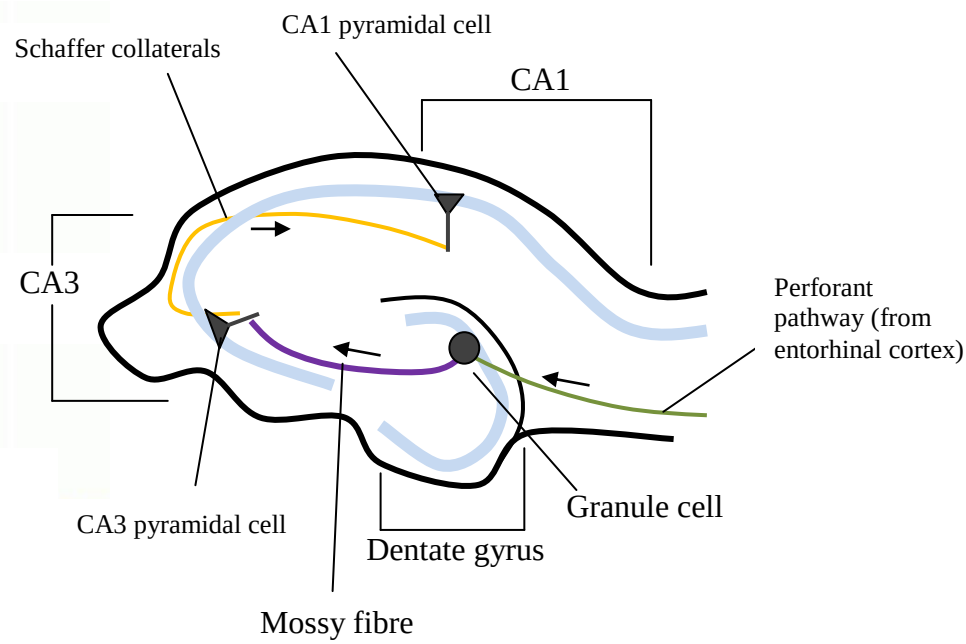

Figure 1.4 The schematic diagram of a rat hippocampal networks in the hippocampus.

Another important pathway is Schaffer collateral pathway. The axons are derived either from the neurons of the same hippocampus (ipsilateral) or from the other hippocampus from another hemisphere (contralateral). The pathway that is derived from the contralateral part is called commissural fibres. It originates from CA3 pyramidal neurons and ends on proximal CA1 dendrites in the stratum radiatum (Garelick & Kennedy, 2011). This pathway has functional roles in NMDA receptor-dependent long-term potentiation and long-term depression.

1.4 Glutamatergic System

Glutamic acid is the major excitatory neurotransmitter in central nervous system (Takamori *et. al*, 2002). There are three types of postsynaptic glutamate receptors that have been identified on the basis of binding affinities for prototypical ligands namely kainate, α-amino-3-hydroxy-5-methyl-4-isoxalone propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors. There are several processes that have been mediated by glutamatergic neurons including the encoding of information, the formation and retrieval of memories, spatial recognition and the maintenance of consciousness (McEntee and Crook, 1993; Kostandy, 2012).

Excessive excitation of glutamate receptors has been associated with the pathophysiology of hypoxic injury, hypoglycemia, stroke and epilepsy. Kristian and Siesjo (1998) reported that there is a strong association between injury and excessive excitation of NMDA receptor, which gates the entry into neurons of potentially toxic levels of Ca^{2+} (Greenhill and Jones, 2010).

1.5 The Effects of Acute and Chronic Exposure

Doses are classified by the integration of exposure concentration over time and this will produce certain effects. This approach is useful in assessing chronic effects, where the time period is long (days or even years). This approach is also useful where there is a large range of possible exposure concentration. Thus, chronic effects are dependent on two parameters: time and concentration. The period of administration of the test substance to animals will depend on the expected period of the clinical use (Table 1.2).

In acute effects, the time component of the dose is not important but the concentration of the dose plays an important role. Effects may be local or systemic. Local effects may appear at skin, eyes and respiratory tract. It is often difficult to find a dose or exposure concentration – effect relationship. Additionally, with the systemic effects, the whole body or a number of organs may get affected (Hasani-Ranjbar *et al*., 2009).

Table 1.2 The commonly used ranges of administration periods (Pacific, 1993; Hasani-Ranjbar *et al*., 2009).

Expected period of clinical use	**Administration period for the toxicity study in animal**
Single administration or repeated administration for less than one week	2 weeks to 1 month
Repeated administration, between one week to four weeks	4 weeks to 3 months
Repeated administration, between one to six months	3 to 6 months
Long-term repeated administration for more than six months	9 to 12 months

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1.5.1 Neurotoxicity

Neurotoxicity is described as a tendency of substances (neurotoxins), conditions or states to modify the regular activity of nervous system. This condition will disrupt or kill neurons. Neurotoxicity can result from exposure to drug therapies and certain chemical compounds (Boos *et al*., 2010). Exposure to certain poisons, which in this case neurotoxins, will alter the normal activities of the nervous system which then cause damage to the neural tissue. In relation to the exposure, symptoms may appear immediately or may be delayed. Neurotoxin is defined to describe a substance, state or condition that damages the neurons. The presence of neurocognitive deficits alone is not considered sufficient evidence of neurotoxicity. This is because the existence of many substances may impair neurocognitive performance without harming neurons. This is probably the result from the direct action of the substance, with the impairment and neurocognitive deficits being temporary, and resolving when the substance is metabolised from the body.

There are some naturally occurring brain toxins that lead to neurotoxicity as a result of excessive dosage. They are β amyloid (Aβ), glutamate and oxygen radicals. In high concentrations, they may lead to neurotoxicity and death (apoptosis). There are a few symptoms that result from the cell death such as loss of motor control, cognitive deterioration and autonomic nervous system dysfunction. In addition, neurotoxicity has been found to be the main cause of neurodegenerative diseases such as Alzheimer's disease (Marchesi, 2012).

1.5.2 Behavioural Toxicity

It is common for medicinal drug users to encounter the behavioural toxicity and evidence shows that there are adverse effects and this prevents them from performing everyday activities in a normal manner. Empirical studies indicate that behavioural toxicity can differ widely between individual drugs. This depends on differences in dose, dosing regimen, duration of treatment, pharmacokinetics or mechanisms of actions (Ramaekers, 1998). For instance, at low levels, lead intoxication will lead to aggressive behaviour as well as learning disabilities (Nigg *et al*., 2008). While manganese, at high levels of exposure will contribute to Parkinsonism and linked to violent behaviour (Aschner *et al*., 2009). Hence, behavioural toxicity assessments need to be conducted to confirm that a particular drug's potential effects on behaviour.

1.6 Action Potential

Action potential is a membrane potential of cells that rapidly rises and fall in short-lasting time by the consistent trajectory. Action potential occurs at excitable cells such as neuronal cells, muscle cells, endocrine cells and some plant cells. In neurons, action potential is involved in cell-to-cell communication. It is often called nerve impulses or spike. Action potential is generated by voltage-gated ion channels which are embedded in the plasma membrane. The channels are shut when membrane potential is near the resting membrane potential of the cell. However, it will open rapidly if the membrane potential increases to a defined precise threshold. When the channel opens, there will be an inward flow of Na^+ which will further rise in the membrane potential. This will lead to more channels to open and use large increase in electrical current. The rapid influx of Na^+ cause polarity of plasma membrane to reverse and those ion channels

will deactivate. When the Na^+ channel closes, Na^+ no longer enter the neuron but the ions transported outside instead. At that stage, potassium ion channels will be activated and produce the outward currents of K^+ which then returns to the resting state. After action potential occurs, the transient negative shift called afterhyperpolarisation or refractory period will take place. This prevents an action potential from traveling back to the way it came from (Lodish et al., 2000; Barnett and Larkman, 2007). The action potential is summarised in the Figure 1.5.

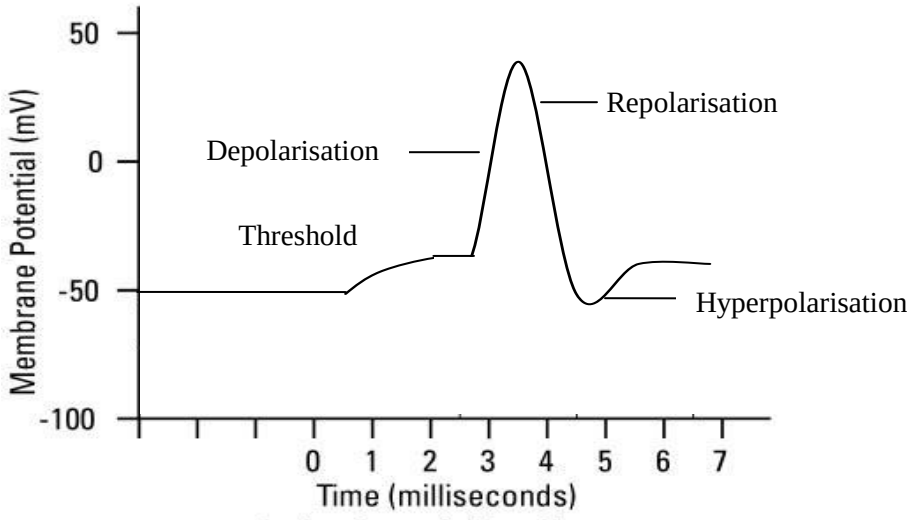

Figure 1.5 A diagram shows a theoretical action potential.

1.7 The EPSP

Excitatory postsynaptic potentials (EPSPs) are the basis in transmitting activities between synaptically connected neurons. The processes where an EPSP causes a postsynaptic cell to fire are important to the operation of neural networks. There are a few factors that influence the efficacy of EPSP-spike coupling: the resting and threshold potential of a postsynaptic cell, the size and the shape of EPSPs. The initiation of spike might also depend on the activation of intrinsic conductance in dendritic as well as in somatic membrane and the site of action potential generation. Multiple intrinsic ionic conductances may be activated by subthreshold EPSPs. The activation of inward currents tends to amplify the EPSPs by increasing the amplitude and prolonging the decay. The axosomatically located sodium channels largely influences the EPSPs amplification in pyramidal cells of hippocampus and neocortex (Andreasen and Lambert, 1999). For instance, in pyramidal cells, EPSP amplification augments the efficacy of dendritic synapses and thus, compensating for an attenuation of distal events (Andreasen and Lambert, 1998; Valeeva *et al*., 2010).

A study by Fricker and Miles (2000) showed that temporal precision of action potential generation by EPSPs depends on the balance of inward and outward currents that are active and near to threshold. Small EPSPs evoked from sub-threshold potentials in hippocampal interneurons initiate action potentials with precise timing and short latencies. However, in pyramidal cells, firing is initiated with longer and more variable delays.

1.8 Learning and Memory

Memory is a behavioural change caused by an experience, while learning is a process for gaining memory. There are several types of memories, such as those concerned with the events and facts and available to our consciousness. This type of memory is called declarative memory. Another type of memory which is not available to our consciousness is called nondeclarative memory. This memory requires the use of a previously learned skill. Declarative memory and nondeclarative memory are independent. Because of this fact, scientists believe that there are separate mechanisms as well as separate regions in the brain that are involved in the mechanisms. Declarative memory occurs in the cerebrum and hippocampus while nondeclarative memory occurs in amygdala, cerebellum and striatum (Okano *et al*., 2000). Synapse is a popular site for memory storage where nerve cells communicate (Kandel, 2013). In other words, if there is a change in transmission efficacy, at the synapse or similarly called synaptic plasticity, it should be considered to be the cause of memory.

The activation of NMDA receptors in various ways can cause either long-term potentiation (LTP) or long term-depression (LTD). These types of synaptic plasticity might represent certain ways of encoding memories in the brain. The size and nature of the changes of synaptic strength are highly regulated process in learning and memory. There are several ways to alter synaptic strength. The ultimate result can be affected by the probability of transmitter release from activated presynaptic terminals, a change in the number of receptor, a change in the size of current produced by each receptor at presynaptic site, a change in the excitability of dendritic membrane and/or changes in