BEHAVIOURAL EFFECTS AND BRAIN ACTIVITY OF MITRAGYNINE FROM *MITRAGYNA SPECIOSA* KORTH (KRATOM) ON LEARNING AND MEMORY FUNCTIONS IN RAT

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by

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytriptamine
A_{595}	Absorbance at 595 nm
Acetyl-CoA	Acetyl coenzyme A
ACh	Acetylcholine
AChE	Acetylcholinesterase
ALT	Alanine transaminase
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	Analysis of variance
AP	Anterior-posterior
AST	Aspartate transaminase
ATPase	Adenosinetriphosphatase
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
BuChE	Butyrylcholinesterase
Ca^{2+}	Calcium ion
CaM	Calcium sensor calmodulin
CaMKII	calcium/calmodulin-dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CB	Cannabinoid receptors
ChAT	Choline acetyltransferase
cm	Centimetre
CNS	Central nervous system
COOH	Carboxyl group
COX	Cyclooxygenase
CREB	Cyclic AMP response element binding protein
DBB	Diagonal band of Broca
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)

EEG	Electroencephalogram
g	Gram
G	Group
GABA	Gamma-aminobutyric acid
GTP	Guanosine triphosphate
H_2O_2	Hydrogen peroxide
HDBB	Horizontal limb of the diagonal band of Broca
HPLC	High-performance liquid chromatography
h	Hour
Hz	Hertz
i.p	Intraperitoneal
Iso-OMPA	Tetraisopropyl pyrophosphoramide
JNK3	c-Jun N-terminal kinase 3
kg	Kilogram
LTD	Long-term depression
LTM	Long-term memory
LTP	Long-term potentiation
M	Molarity
mA	Miliampere
mAChR	Muscarinic acetylcholine receptor
mg	Miligram
Mg^+	Magnesium ion
min	Minutes
Mit 1	Mitragynine-treated group at 1 mg/kg
Mit 10	Mitragynine-treated group at 10 mg/kg
Mit 5	Mitragynine-treated group at 5 mg/kg
ML	Mediolateral
ml	Mililitre
mm	Milimetre

Mor	Morphine-treated group at 5 mg/kg
Na^+	Sodium ion
nAChR	Nicotinic acetylcholine receptor
NBM	nucleus basalis magnocellularis
nm	Nanometre
NMDA	*N*-methyl-D-aspartate
OD	Optical density
Oxo	Oxotremorine
Phy	Physostigmine
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein kinase G
s	Second
SEM	Standard error mean
STM	Short-term memory
USM	Universiti Sains Malaysia
V	Ventral
Veh	Vehicle-treated group
VDBB	Vertical limb of the diagonal band of Broca
µl	Microlitre
µm	Micrometre
µV	Microvolt

LIST OF SYMBOLS

β	Beta
α	Alpha
γ	Gamma
δ	Delta
ε	Epsilon
μ	Mu
κ	Kappa
°C	Degree celcius
%	Percentage
θ	Theta
<	Less than
±	Plus minus
~	Approximately

LIST OF APPENDICES

KESAN-KESAN TINGKAH LAKU DAN AKTIVITI OTAK OLEH MITRAGININA DARIPADA *MITRAGYNA SPECIOSA* KORTH (KRATOM) KE ATAS FUNGSI PEMBELAJARAN DAN MEMORI DALAM TIKUS

ABSTRAK

Mitraginina, sebatian alkaloid utama daripada *Mitragyna speciosa* Korth telah dikaji secara meluas untuk kesan-kesan farmakologinya. Walau bagaimanapun, sedikit maklumat yang diketahui tentang kesannya terhadap fungsi pembelajaran dan memori. Dalam penyelidikan ini, kesan-kesan akut mitraginina terhadap peringkat-peringkat yang berlainan dalam proses pembelajaran dan memori telah dikaji. Pemberian pra-latihan, pasca-latihan dan pra-ujian mitraginina (1, 5 dan 10 mg/kg) menjejaskan pemerolehan, pengukuhan dan dapatan semula memori dalam prosedur penghindaran pasif, ke tahap yang sama seperti morfina. Penyelidikan ini seterusnya mengkaji jika kesan mitraginina adalah bergantung kepada prosedur atau secara umum ke atas prosedur tingkah laku yang lain. Hasil kajian menunjukkan bahawa mitraginina (5 dan 10 mg/kg) menyebabkan defisit pembelajaran spasial dan menjejaskan memori rujukan dalam prosedur berselirat air Morris. Kemudian, kesan-kesan kronik mitraginina (28 hari) ke atas fungsi pembelajaran dan memori dinilai. Hasil kajian menunjukkan bahawa kronik mitraginina menjejaskan memori dapatan semula prosedur penghindaran pasif semasa penyisihan awal dan ketika abstinens. Kesan-kesan mitraginina dinilai lagi menggunakan prosedur pengiktirafan objek novel dalam haiwan yang sama. Hasil kajian menunjukkan bahawa haiwan mampu mendiskriminasi antara objek novel dan biasa tetapi keupayaan untuk mengingati objek yang telah dihadapi sebelum ini telah dilemahkan dalam haiwan yang dirawat dengan dos mitraginina yang tinggi (10 mg/kg). Keputusan menunjukkan bahawa

dos mitraginina yang tinggi diperlukan untuk mengekalkan kesan-kesan kerosakan memori oleh mitraginina dalam tugasan pembelajaran yang baru ketika abstinens. Kesan-kesan mitraginina ke atas aktiviti otak dinilai lagi pada haiwan-haiwan yang dirawat secara kronik untuk 28 hari. Mitraginina menyebabkan corak EEG yang berbeza pada kedua-dua tisu kortikal (frontal dan neokorteks) dan hipokampus. Pendedahan berulang kepada dos mitraginina yang sama pada 10 mg/kg menyebabkan peningkatan dalam kuasa delta dan penurunan dalam kuasa alfa di kedua-dua korteks frontal dan neokorteks. Sebaliknya, pendedahan berulang kepada mitraginina menurunkan kuasa delta tanpa mengira dos tetapi hanya mitraginina pada 5 dan 10 mg/kg menurunkan kuasa alfa di hipokampus. Perubahan dalam EEG ini mencadangkan bahawa mitraginina mampu mencabar kestabilan rangkaian kawasan setempat. Di samping itu, pemberian nalokson, antagonis opioid (1.0 mg/kg) dan bikukulina, antagonis $GABA_A$ (0.125 mg/kg) pasca-latihan memulihkan memori yang terjejas disebabkan oleh mitraginina, mencadangkan penglibatan sistem opioidergik dan GABAergik dalam modulasi kesan-kesan mitraginina ke atas fungsi pembelajaran dan memori. Dalam prosedur berselirat air Morris, pemberian agonis kolinergik, oksotrimorina (0.1 mg/kg) pasca-latihan, memulihkan defisit pembelajaran spasial yang disebabkan oleh mitraginina (5 dan 10 mg/kg). Pemberian fisostigmina (0.1 mg/kg), antikolinesterase hanya memulihkan kesan kerosakan oleh mitraginina terhadap pembelajaran spasial pada 5 mg/kg tetapi tidak pada dos yang lebih tinggi (10 mg/kg). Keputusan ini mungkin menunjukkan bahawa defisit pembelajaran spasial yang disebabkan oleh mitraginina adalah berkolerasi dengan pengurangan paras asetilkolina di kawasan-kawasan otak yang berkaitan dengan memori. Pra-rawatan mitraginina selama 12 hari berturut-turut membawa kepada perbezaan aktiviti kolinesterase di dalam otak. Tiada perbezaan dalam aktiviti

asetilkolinesterase (AChE) dilihat dalam korteks frontal. Lebihan korteks yang terdiri daripada tisu kortikal utama menunjukkan penurunan manakala hipokampus menunjukkan peningkatan aktiviti AChE dalam kumpulan yang telah dirawat dengan mitraginina pada 10 mg/kg. Secara keseluruhannya, boleh disimpulkan bahawa penggunaan mitraginina boleh memberi kesan kepada fungsi pembelajaran dan memori seperti yang ditunjukkan oleh prestasi pencapaian tingkah laku yang terjejas dan perubahan dalam aktiviti otak.

BEHAVIOURAL EFFECTS AND BRAIN ACTIVITY OF MITRAGYNINE FROM *MITRAGYNA SPECIOSA* KORTH (KRATOM) ON LEARNING AND MEMORY FUNCTIONS IN RAT

ABSTRACT

Mitragynine, the major alkaloid from *Mitragyna speciosa* Korth has been widely studied for its pharmacological effects. However, little is known about its effects on learning and memory functions. In present study, the acute effects of mitragynine on different stages of learning and memory processes were investigated. Pre-training, post-training and pre-test administration of mitragynine (1, 5 and 10 mg/kg) impaired the acquisition, memory consolidation and retrieval of the passive avoidance task, to a similar degree as morphine. The study further investigated if the effects of mitragynine are either task-dependent or common to other behavioural tasks. The results showed that mitragynine (5 and 10 mg/kg) caused spatial learning deficits and impaired reference memory in Morris water maze task. Then, chronic effects of mitragynine (28 days) on learning and memory functions were assessed. Results demonstrated that chronic mitragynine impaired memory retrieval of the passive avoidance task during early withdrawal and abstinence. Effects of mitragynine were further evaluated using novel object recognition task in the same treated-animals. The results indicated that animals were able to discriminate between the novel and familiar objects but the ability to remember the previously encountered object was attenuated in the animals treated with high dose of mitragynine (10 mg/kg). The results suggested that high dose of mitragynine is required to maintain the memory-impairing effects of mitragynine in a new learning task during abstinence. The effects of mitragynine on brain activity were further evaluated in

chronically-treated animals for 28 days. Mitragynine caused dissociative patterns in EEG of both cortical tissues (frontal and neocortex) and hippocampus. Repeated exposure to equal dose of mitragynine at 10 mg/kg caused an increase in delta power and a decrease in alpha power in both frontal cortex and neocortex. On the contrary, repeated exposure to mitragynine regardless of the doses decreased the delta power but only mitragynine at 5 and 10 mg/kg decreased the alpha power in the hippocampus. These changes in EEG may suggest that mitragynine was able to challenge the local network stability. In addition, post-training administration of naloxone, an opioid antagonist (1.0 mg/kg) and bicuculline, $GABA_A$ antagonist (0.125 mg/kg) improved memory impairment induced by mitragynine, suggesting the participation of the opioidergic and GABAergic systems in modulating the effects of mitragynine in learning and memory functions. In Morris water maze task, post-training administration of cholinergic agonist, oxotremorine (0.1 mg/kg), improved the spatial learning deficit induced by mitragynine (5 and 10 mg/kg). The administration of physostigmine (0.1 mg/kg), an anticholinesterase only reversed the impairing effects of mitragynine on spatial learning at 5 mg /kg but not at higher dose (10 mg/kg). These results may indicate that mitragynine-induced spatial learning deficit is correlated with the depletion of acetylcholine level in memory-associated brain regions. Pre-treatment of mitragynine for 12 consecutive days led to differential cholinesterase activities in brain. No difference in acetylcholinesterase (AChE) activity was observed in frontal cortex. Remaining cortex which consists of major cortical tissues showed a decrease whilst hippocampus showed enhanced AChE activity in mitragynine-treated group at 10 mg/kg. Taken together, it can be concluded that mitragynine consumption can affect learning and memory functions as shown by the impaired behavioural performances and changes in brain activity.

CHAPTER ONE

INTRODUCTION

1.1 Learning and memory

Behaviour involves a continuous flow of information between several interacting brain systems. The systems process the information and the output ultimately controls the behaviour, either directly or indirectly. Under certain circumstances, the information being processed may change the neural systems, which later alter the processing of similar information in future occasions, and eventually change the corresponding output of the system (White and McDonald, 2002; Shohamy and Adcock; 2010). The changed output causes an alteration in behaviour which attributes to a process called 'learning' and eventually leads to the inference of the existence of a 'memory'.

1.1.1 Stages of learning and memory

'Memory' can be defined as the ability to remember previously learned information or past experiences (Richter-Levin and Akirav, 2000) and it can be measured as a change in behaviour some time after learning (Abel and Lattal, 2001). Memory has always been confined to the idea of how new information is stored in the brain and how knowledge is organized as a cognitive process (White and MacDonald, 2002). Basically, learning and memory processes consist of three different phases *i.e.* memory formation or also known as acquisition, consolidation and retrieval or recall of the previously learned information (Izquierdo, 1989; Deiana et al., 2011). Acquisition refers to the process on how the new information is

encoded whilst the consolidation refers to the state where the newly acquired information that is labile and fragile undergoes a lingering process, becoming stronger and resilient over time. Retrieval can be defined as a process of recall of the stored information (Medina et al., 2008).

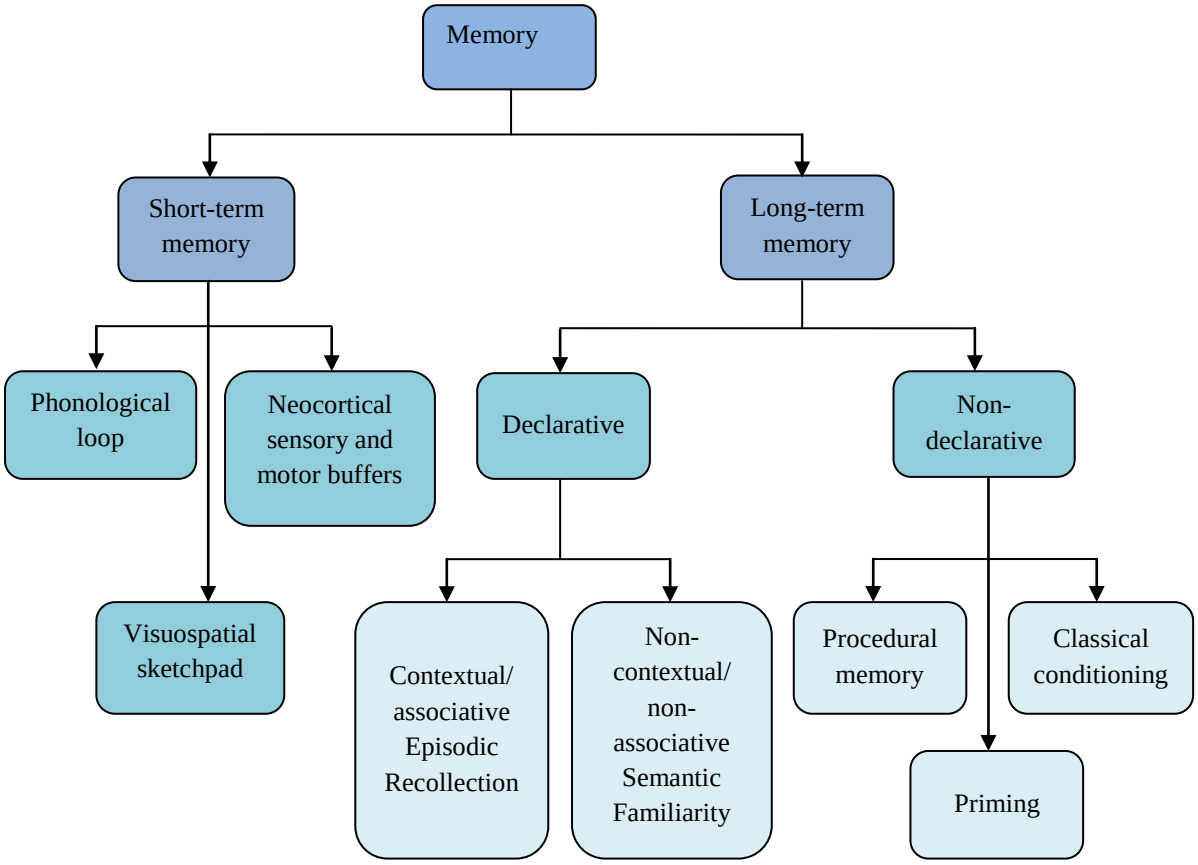

Figure 1.1: The traditional taxonomy of memory systems (Edited from Bird and Burgess, 2008).

1.1.2 Types of memory

Classifications of memories are based on their duration (short- or long-term) and their nature which could be archival (short- or long-term memory) or transient (working memory) (Deiana et al., 2011; Izquierdo et al., 1999). In mid 1980s, the term declarative and 'non-declarative' were introduced with declarative memory refers to one memory system and non-declarative memory refers to other additional

memory systems (Squire, 2004; Figure 1.1). Declarative memory refers to the capacity of conscious recollection of facts or events that can be brought to mind and described verbally. This kind of memory is often impaired in amnesia and is dependent on structures in the medial temporal lobe and midline diencephalon. Declarative memory allows the retained information to be compared and contrasted and thus, supports the encoding of memories in terms of relationships among multiple items and events. In contrast, non-declarative memory can be referred as unconscious recollection of experiences such as habits and skills that are expressed in performances. This kind of memory occurs due to modifications within specialized performance systems and is revealed via reactivation of the systems within which the process of learning occurred (Squire, 2004; Deiana et al., 2011).

Declarative memory can be subdivided into semantic and episodic memory. The former constitutes the memory of meanings, facts, understanding and concept-based knowledge, and the latter implies the ability to re-collect an event in the environment in which it originally occurred and involves detailed elements (what), location (where) and temporal occurrence (when). Spatial memory is usually considered in the context of episodic memory and it implies the ability to attain information about one's environment and its spatial orientation. Construction of cognitive maps is based on the orientation in space as indicated by landmark cues. Establishment of these cognitive maps follows either egocentric or allocentric strategies. Egocentric involves self-to-object representational system whilst allocentric involves relationship of oneself to three-dimensional arrangements of objects in the environment (Deiana et al., 2011).

McGaugh (1966) has introduced a 'three memory trace systems' concept which consists of immediate memory, short-term memory (STM) and the one that consolidates slowly and is relatively permanent (long-term memory, LTM). Immediate memory or known as working memory lasts for seconds or a few minutes. It depends primarily on the electrical activity of prefrontal cortex cells which are in connections with other brain regions. Basically, this type of memory works as an online system since it only persists as long as the electrical activity persists (Izquierdo et al., 1999). Meanwhile, STM is defined as a memory that develops within a few seconds or minutes but lasts for several hours, while LTM has been regarded as memory which persists at least for 24 hours. This persistence of LTM requires gene activation and protein synthesis which differentiates it from STM. Another major difference between the STM and LTM is that the latter is more susceptible to extinction (Izquierdo et al., 1999).

Biochemical investigations led to a finding that showed in CA1 of the hippocampus that both STM and LTM are dependent on the integrity of the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA), *N*-methyl-D-aspartate (NMDA) and metabotropic glutamate receptors as well as their modulation by cholinergic muscarinic, β-adrenergic (stimulant) and $GABA_A$ receptors (inhibitory). However, only LTM is dependent upon post-training protein kinase C (PKC), protein kinase G (PKG) or calcium/calmodulin-dependent protein kinase II (CaMKII) activities. Meanwhile, protein kinase A (PKA) shows a distinct influence on each STM and LTM whereby, it modulates the STM between zero and 90 minutes after training and LTM at zero and after 180 minutes after the training, but not in between those two peaks. In LTM, PKA is involved in the phosphorylation of the nuclear

transcription factor $CREB_1$ (cAMP response element binding protein) (Izquierdo et al, 1999; 2004).

On the contrary, it seems no involvement of NMDA receptors in the STM formation in entorhinal cortex or elsewhere. In addition, the role of PKC and PKA are both necessary for the formation of STM and LTM in entorhinal cortex (Izquierdo et al., 1999).

1.1.3 Mechanisms of learning and memory

Learning and memory are basically supported by synaptic plasticity in the brain. Synaptic plasticity can be referred as any lasting upregulation or downregulation of synaptic strength including long-term potentiation (LTP), long-term depression (LTD) or depotentiation, and the changes of the synaptic strength can occur as a result of certain types of learning (Morgado-Bernal, 2011; Johnston et al., 2003; Martin and Morris, 2002). It occurs at the dendritic spines, the major site of excitatory synaptic transmission in the vertebrate brain. Studies on the initiation and the maintenance of synaptic plasticity, particularly in CA1 pyramidal neurons of the hippocampus, have shown that certain types of learning can induce an increase in the number or morphological changes of the dendritic spines leading to a new or strengthen existing synapses that may form the principle basis of memory (Morgado-Bernal, 2011; Penzes and Jones, 2008).

Pharmacological manipulations have succeeded in establishing the major molecular mechanisms of memory processes. Initially, activation of relevant synapses causes a pre-synaptic release of glutamate which then activates the ionotropic AMPA receptors (AMPAr). Activation of this AMPAr leads to an influx of Ca^{2+} into the post-synaptic neuron thus depolarization occurs. Within a few

seconds, the local post-synaptic depolarization activates the NMDA receptors causing the removal of Mg^{+} which blocks the channel of NMDA receptors and thus giving rise to a massive influx of Ca^{2+} into the post-synaptic neuron. Consequently, CaMKII, PKC and calcineurin are activated. Adenylyl cyclase is also activated by the influx of Ca^{2+} or other modulatory inputs via G-protein-coupled receptors. As a result, cyclic adenosine monophosphate (cAMP) level is increased and leads to activation of PKA that eventually regulates the synthesis and availability of new proteins via activation of CREB in the nucleus (Morgado-Bernal, 2011; Abel and Lattal, 2001; Maren and Baudry, 1995; Figure 1.2).

In this complex process, Ca^{2+} binds to calcium sensor calmodulin (CaM) and activates different CaMKs causing morphological changes of the cytoskeleton of the neuron, for instance new dendritic spines or persistence enlargement of its head. These morphological changes are strongly dependent on the protein synthesis, as well as brain derived neurotrophic factor (BDNF) actions (Morgado-Bernal, 2011). The formation and the maintenance of new dendritic spines provide a persistent structural change upon which a long-term memory is established.

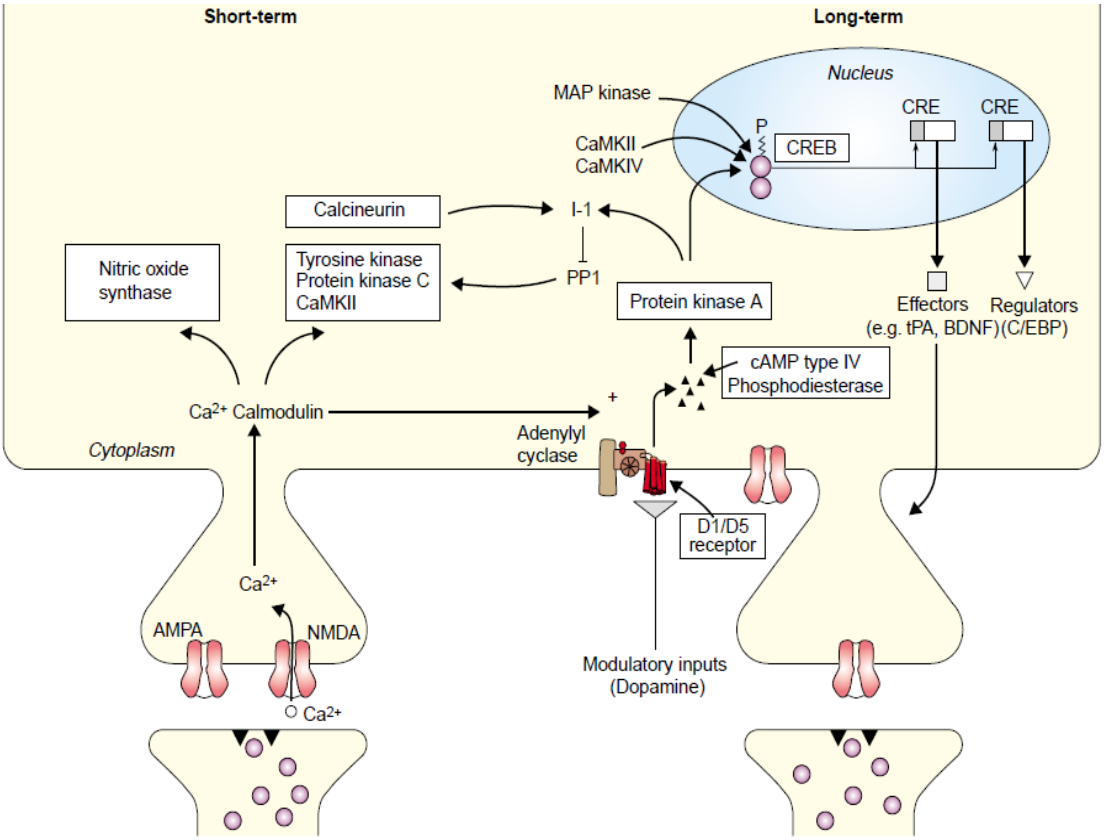

Figure 1.2: Molecular events underlie the early and late phases of long-term potentiation (Adapted from Abel and Lattal, 2001).

1.1.4 Memory modulatory mechanisms in the brain

Extensive research has been carried out to investigate the neurobiological processes and systems that contribute to the differences in memory strength. Modulatory systems not only affect the neurobiological processes of newly acquired information but also other mnemonic processes including working memory, memory recall and memory extinction (Roozendaal and McGaugh, 2011; Cahill and McGaugh, 1996; Figure 1.3).

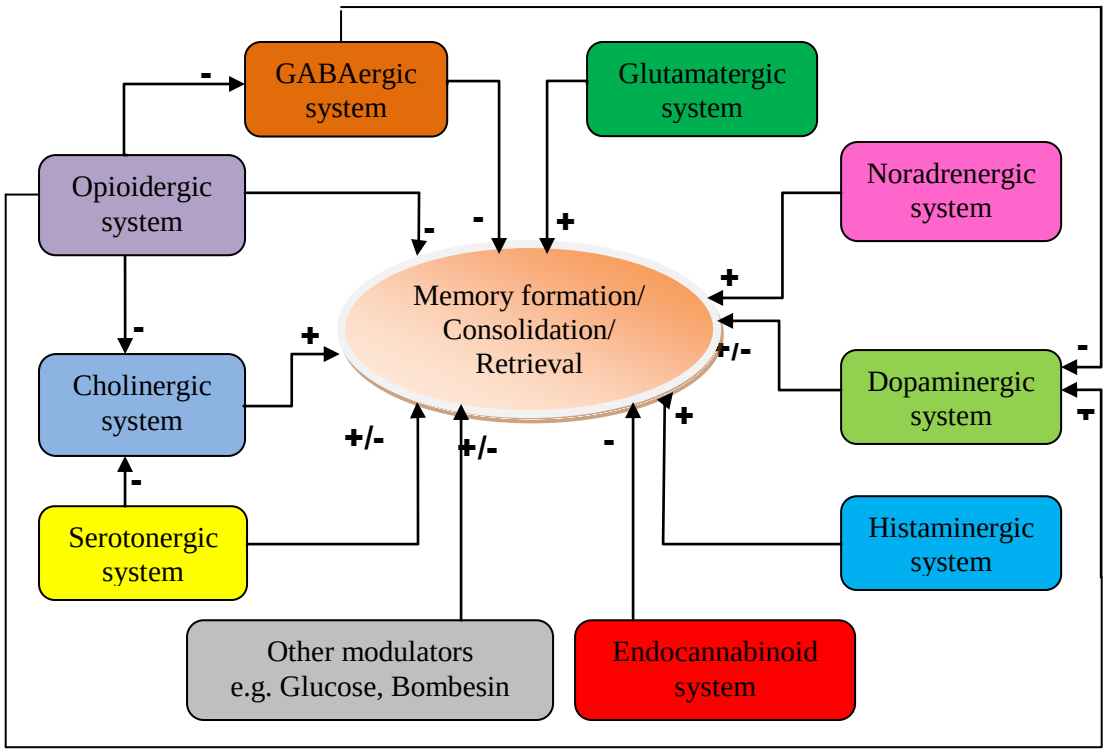

Figure 1.3: Modulatory systems of learning and memory functions in the brain.

1.1.4(a) GABA

Gamma (γ)-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in brain that can act on three receptor subtypes: $GABA_A$, $GABA_B$ and $GABA_C$. Both $GABA_A$ and $GABA_B$ are confined to the central nervous system while $GABA_C$ is specific for retina (Lanctot et al., 2004). $GABA_A$ receptor is a ligand-gated ion channel that can be found at both pre- and post-synaptic neurons. Upon binding with $GABA_A$ agonist, flux of chloride ions increase leading to neurons hyperpolarization and thus reduce the synaptic transmission of dopamine, serotonin and acetylcholine (ACh). Other than its GABA-agonist binding site, $GABA_A$ receptor has specific sites for benzodiazepines, barbiturates, steroids and ethanol (Lanctot et al., 2004). Therefore, any interference in individual binding sites or at the whole receptor unit may change the modulatory effect of GABA on other neuronal

systems. Meanwhile, $GABA_B$ is linked to G-proteins and second-messenger system that mediates calcium or potassium channels which in turn generating the slow inhibitory post-synaptic potentials in several brain regions (Lanctot et al., 2004; Staubli et al., 1999).

Bicuculline, $GABA_A$ antagonist has been proven pharmacologically to facilitate the memory formation (Izquierdo et al., 1992; Izquierdo and McGaugh, 2000) while picrotoxin acts by blocking the chloride channel of $GABA_A$ receptors, facilitates both memory formation and extinction (McGaugh et al., 1990; Izquierdo et al., 2004). On the contrary, muscimol ($GABA_A$ agonist) that mimics the action of GABA disrupts the memory performance and tiagabine, impairs the spatial learning via inhibition of the transport of GABA away from the synaptic cleft (Izquierdo et al., 2004).

The importance of GABAergic system is further illustrated in a study using PWZ-029, an α_5 $GABA_A$ selective inverse agonist. It was found that PWZ-029 administration was able to improve memory in object recognition task after 24-hours delay in normal and scopolamine-treated rats. However, PWZ-029 seemed to be ineffective to improve the performance in Morris water maze task in either normal or scopolamine-treated rats (Milic et al., 2013).

1.1.4(b) Monoaminergic system

The role of dopaminergic, noradrenergic and serotonergic systems in modulating the learning and memory processes in various memory-associated brain regions including hippocampus, basolateral amygdala, entorhinal and parietal cortex has been well established (Roozendaal and McGaugh, 2011; O'Carroll et al., 2006; Izquierdo and McGaugh; 2000). Pharmacological manipulation has shown that

dopamine uptake inhibition improves the acquisition of the inhibitory avoidance and increases the hippocampal ACh release (Nail-Boucherie et al., 1998). However, studies on dopaminergic modulation on learning and memory showed contrasting results which may be due to the activation of different receptor subtypes and different experimental design. For instance, activation of D1 receptor caused an increase but activation of D2 receptor led to a decrease in the cAMP production. Meanwhile, D3 receptor was not related to the adenylate cyclase and was not affected by GTP which modulates the binding associated with D1 and D2 receptor (Snyder, 1992; Sokoloff et al., 1990; Zarrindast, 2006). Administration of D1 receptor agonist resulted in enhanced cognitive performance in Morris water maze task in rats (Hersi et al., 1995) or had no effects on radial-arm maze learning (Wilkerson and Levin, 1999). Findings by Zarrindast et al. (1996) demonstrated that administration of low and high doses of apomorphine, a mixed D1/D2 dopamine receptor agonist in mice, improved or impaired memory retrieval in the active avoidance task, respectively.

Noradrenergic neurons originated from locus coerulus innervate many brain regions, ranging from the spinal cord and cerebellum to the forebrain (Sirvio and MacDonald, 1999). The modulatory effects of noradrenergic receptors on learning and memory may be mediated via α_1-, α_2-, β_1-, and β_2-adrenoceptors (Zarrindast, 2006). Previous study reported that activation of β-noradrenergic receptor, 3 to 4 hours after induction can help to maintain LTP in conscious rats (Straube and Frey, 2003) and thus enhancing the memory. Local infusion of norepinephrine into the basolateral amygdala of rats also enhanced the consolidation of two different forms of contextual fear conditioning (LaLumiere et al., 2003). In addition, previous study

stated the memory-enhancing effects were mediated by the β-adrenoceptors whilst α_1-adrenoceptors inhibited memory consolidation (Gibbs and Summers, 2001).

Serotonin (5-hydroxytriptamine, 5-HT) is a biogenic amine that is implicated in various physiological functions such as sleep, pain perception, memory and mood control (Polter and Li, 2010; Meneses and Perez-Garcia, 2007; Zarrindast, 2006; Buhot et al., 2000; McEntee and Crook, 1991). The central nervous system (CNS) effects of the serotonin are mediated by 5-HT receptor that can be classified into seven main classes; 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 which are based on structural, transductional and operational features (Hannon and Hoyer, 2008; Hoyer et al., 2002). Some 5-HT receptors can be further subdivided into several subtypes (Zarrindast, 2006). Activation of 5-HT receptors lead to diverse effects on learning and memory processes and usually, activation of 5-HT_{1A} receptors promote an enhancement in memory consolidation and retrieval (Izquierdo et al., 2004). In another study, impairment of short-term memory but not long-term memory was observed due to serotonin depletion after intracerebroventricular injection of serotonin neurotoxin and serotonin synthesis inhibitor in rats (Hritcu et al., 2007).

A number of studies reported that 5-HT is potentially interacting with other neural systems to contribute to a particular function (Lesch and Waider, 2012; Ciranna, 2006). In entorhinal cortex, 5-HT activates 5-HT_3 receptors on the GABAergic neuron causing an inhibition of the cholinergic function (Ramirez et al., 1996). 5-HT_{1A} antagonist prevents the impairment of spatial learning induced by intrahippocampal scopolamine, plausibly via favorable actions on other excitatory neurotransmitters (Carli et al., 1995a). In another studies, activation of 5-HT_{1A} receptors in the hippocampal CA1 region causes impairment in spatial but not visual

discrimination in rats (Carli et al., 1995b) whilst blockade of 5-HT_{2A} receptors in the medial prefrontal cortex impairs the recognition memory in spontaneous novelty preference task (Bekinschtein et al., 2013).

Taken together, dopaminergic, noradrenergic and serotonergic play a pivotal role in modulating the learning and memory, and it seems possible that these neural systems work with other neural systems to modulate the learning and memory processes.

1.1.4(c) Cholinergic system

The involvement of cholinergic system in learning and memory processes has gained a great interest among researchers (Blokland, 1996; Robinson et al., 2011; Deiana et al., 2011; Miranda et al., 2003) and numerous studies have confirmed the participation of cholinergic system in modulating learning and memory processes (Easton et al., 2012; Pych et al., 2005; Farr et al., 2000). In rodent, there are at least six cholinergic cell groups that project to different brain areas. Both medial septal nucleus (Ch1) and vertical (VDBB) limb of the diagonal band of Broca (Ch2) from the basal forebrain innervate the hippocampal formation. The horizontal (HDBB) limb of the diagonal band of Broca (Ch3) projects to olfactory bulb and thalamic reticular region whilst nucleus basalis magnocellularis (NBM, Ch4) issuing fibers to the thalamus, cerebral cortex and amygdala. Finally, pedunculopontine (Ch5) and laterodorsal tegmentum (Ch6) which are part of the midbrain regions provide projections to cortical and various thalamic regions. In addition, there are numerous cholinergic fibers that are intrinsic to specific regions, such as striatum (Deiana et al., 2011; Zarrindast, 2006; Everitt and Robbins, 1997; Figure 1.4).

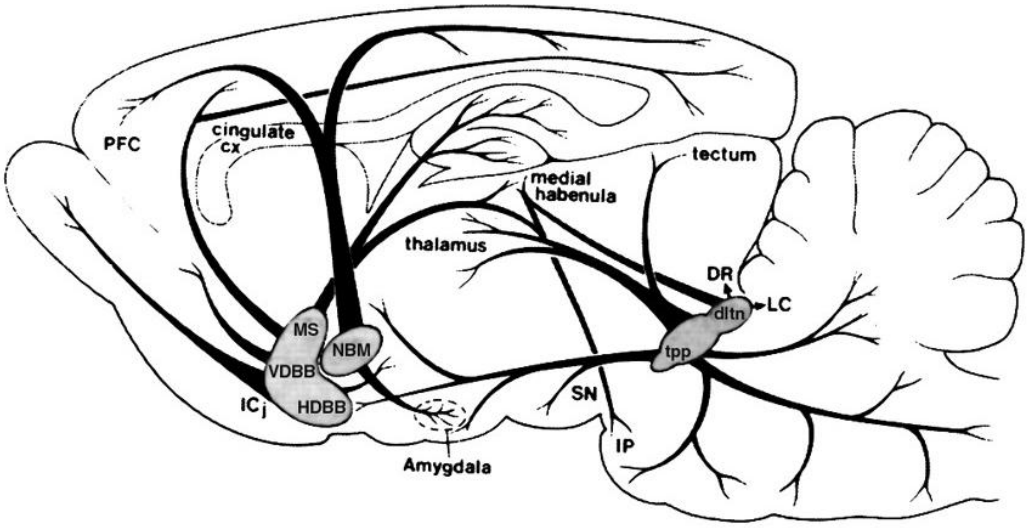

Figure 1.4: Distribution of cholinergic neurons and their projections in the rat brain. Abbreviations: MS, medial septum (cell group Ch1); VDBB, vertical limb nucleus of the diagonal band Broca (cell group Ch2); HDBB, horizontal limb nucleus of the diagonal band Broca (cell group Ch3); NBM, nucleus basalis magnocellularis (cell group Ch4); tpp, pedunculopontine tegmental nucleus (cell group Ch5); dltn, laterodorsal tegmental nucleus (cell group Ch6); PFC, prefrontal cortex; ICj, islands of Calleja; SN, substantia nigra; IP, interpeduncular nucleus; DR, dorsal raphe; LC, locus coerulus (Adapted from Everitt and Robbins, 1997).

ACh is synthesized via the acetylation of choline with acetyl-CoA by enzyme choline acetyltransferase (ChAT) in the nerve ending. Accumulation of ACh in the synaptic vesicle is driven by proton-pumping ATPase. The quantal release of ACh into the synaptic cleft is triggered by depolarization-induced Ca^{2+} flux. ACh will bind to the both pre- and post-synaptic muscarinic (mAChR) and nicotinic (nAChR) receptors (Deiana et al., 2011; Taylor and Brown, 1999). Muscarinic receptors are G-proteins coupled receptors and can be classified into M_1, M_2, M_3, M_4 and M_5. M_1 receptors are widely distributed in CNS particularly in cerebral cortex, hippocampus and amygdala. It is also a dominant mAChR subtype in sympathetic ganglia and exocrine glands. M_2 receptors are abundant in myocardium and M_3 receptors are mainly found in smooth muscle and exocrine glands as well as in cortex,

hippocampus and thalamus. Meanwhile, M_4 receptors showed a scattered appearance in cortex and striatum whilst M_5 receptors are dense in hippocampus, habenula and thalamus (Deiana et al., 2011).

nAChRs are ligand-gated ion channels with pentameric shape consisting of α2-α10, β2-β4, γ, δ and ε (in the adult muscle) subunits that are arranged symmetrically around the central pore. Activation of these receptors leads to a rapid increase in cellular permeability to Na^+ and Ca^{2+}, depolarization and excitation. α2 is scattered in the hippocampus and cortex and α3 is abundant in layer four of cortex, dorsal and ventral thalamus including lateral geniculate. α4 can be found mainly in cortex, thalamus, amygdala and hippocampus whilst α7 is moderately distributed in hippocampus, cortex, cerebellum and brainstem. β2 is dense in thalamus but less in cortex and dorsal hippocampus (Deiana et al., 2011).

Lesions of the cholinergic system or systemic and direct brain injections of cholinergic drugs have been used in many studies for its behavioural effects. Local administration of muscarinic antagonist, scopolamine into the amygdala interfered with the acquisition of the conditioned place preference task (McIntyre et al., 1998). In general, many findings have demonstrated that local administration of cholinergic agonists and antagonists into the striatum, hippocampus and amygdala, enhanced or impaired learning and memory performance in task associated with the neural system, respectively (Gold, 2003).

The availability of the new method called *in vivo* microdialysis/high performance liquid chromatography (HPLC) has enabled the measurement of ACh releases (Schlereth et al., 2006; Gold, 2003; McIntyre et al., 2002; Stancampiano et al., 1999) from the hippocampus of behaving animals. It has been reported that the

increase and decrease in the release of ACh, particularly in hippocampus was correlated with the enhancement and impairment of learning and memory (Gold, 2003). The ACh release may reflect the degree of activation and participation of the cholinergic system in the hippocampus in contributing to the performance of the learning and memory in a particular task.

Cholinesterases *i.e.* acetylcholinesterase and butyrylcholinesterase are pivotal enzymes in cholinergic system. Acetylcholinesterase is present in both central nervous system and peripheral nervous system such as at the parasympathetic and sympathetic ganglia, parasympathetic end-organs, motor end-plates and sweat glands. This enzyme primarily functions to hydrolyze the ACh at these junctions and eventually terminates the cholinergic transmission (Padilla et al., 1999; Pepeu and Giovannini, 2010; Miao et al., 2010). Meanwhile, the role of butyrylcholinesterase is an enigma even though reports indicated that this enzyme plays a role during developmental processes (Zugno et al., 2013). In contrast to acetylcholinesterase, butyrylcholinesterase hydrolyzes both acetylcholine and other esters.

Acetylcholinesterase activity may be an indicator on how the brain is functioning in relation to learning and memory. Isomae et al. (2003) reported that T-82, a novel acetylcholinesterase inhibitor ameliorated the memory impairment induced by acetylcholinergic dysfunction in inhibitory avoidance task. A study found an increase in acetylcholinesterase activity in amnesic brain (Biradar et al., 2012), but in the imaging study of Alzheimer patients, acetylcholinesterase appeared to be reduced in the amygdala (-33%), hippocampus (-14%) and neocortex (-20%) (Shinotoh et al., 2003). Therefore, it is possible that consumption of mitragynine may also leads to the alteration of the cholinesterase activity in the brain.

1.1.4(d) Opioidergic system

Opioid peptides such as enkephalin and β-endorphin are synthesized and released from both peripheral and CNS (Asai et al., 2007; Lin and Pan, 1995; Carrasco et al., 1982). Extensive studies have shown that administration of opioid peptide agonists and antagonists were able to modulate learning and memory processes in a variety of tasks employing both appetitive and aversive motivation, via binding to opioid receptors that are widely distributed in the memory-associated brain regions. Opioid agonists act via G-protein coupled opioid receptors known as μ-, δ- and κ-receptors (Feng et al., 2012; Al-Hasani and Bruchas, 2011; McDonald, 2005). Binding to the opioid receptors causes activation of potassium channels, inhibition of calcium channels, inhibition of adenylyl cyclase which leads to reduced cAMP production as well as inhibition of transmitter release (Dacher and Nugent, 2011; McDonald, 2005), thus affecting the learning and memory processes.

Opioid agonists impair memory, but opioid antagonists have memory-enhancing effects in several paradigms including inhibitory and active avoidance, aversively-motivated Y-maze discrimination, and appetitively-motivated spatial learning (McGaugh, 1989; Zhu et al., 2011). A recent study reported that administration of opioid agonists inhibited the acquisition of passive avoidance task at all stages (Zarrindast et al., 2013). However, the inhibitory effects of opioid agonist on memory could be reversed by naloxone, an opioid antagonist (Izquierdo, 1979). Repeated treatment of morphine impaired the acquisition of both simple appetitive and cued operant learning but withdrawal for five weeks alleviated the impairment. Single morphine exposure caused a disruption in the retrieval of the operant memory, but no effect was observed in rats after five-week withdrawal (Wang et al., 2006).

Opioid agonist may also interact with other neural systems in the brain. It has been reported that opioid agonists inhibited noradrenaline release from the hippocampus, thus suggesting how it regulates the learning and memory processes (Matsumoto et al., 1994). Interaction with the cholinergic system occurs via binding to the opioid receptors located on the cholinergic interneurons causing an inhibition on the acetylcholine release in the brain, thus contributing to the memory impairment (Xi-Geng et al., 2002). It has been reported that administration of opioid agonist, morphine into the medial septum causes a reduction in the ACh release in the hippocampus (Ragozzino and Gold, 1995).

1.1.4(e) Endocannabinoid system

Endocannabinoid receptors, particularly cannabinoid receptors type 1 (CB1) which are widely distributed in the brain has been shown to be involved in learning and memory functions (Moreno and Campolongo, 2014; Peterfi et al., 2012; Castillo et al., 2012; Serrano and Parsons, 2011; De Oliveira Alvares et al., 2008). Cannabinoids have disruptive effects on the learning and memory processes. This was shown through impairment of spatial memory in 8-arm radial maze and inhibition of the acquisition and retrieval of passive avoidance task upon administration of delta 9-tetrahydrocannabinol, the major psychoactive compound of marijuana (Mishima et al., 2001). In another study, spatial learning deficit induced by delta 9-tetrahydrocannabinol was blocked by SR241716A, a specific CB1 antagonist in mice (Da and Takahashi, 2002). Anandamide, the main endogenous ligand, impaired memory consolidation of one-trial inhibitory avoidance in mice but did not affect the retrieval when given before testing (Costanzi et al., 2003). The endocannabinoid system also controls the extinction of the aversive conditioned

responses in rat, presumably in the basolateral amygdala (Marsicano et al., 2002). The amygdala has been characterized by a great presence of CB1 receptors, which are responsible for the local GABAergic transmission (Katona et al., 2001). CB1 cannabinoid receptors may play a pivotal role in endogenous defense against excitotoxicity (Marsicano et al., 2003), in modulation of retrograde signaling in the hippocampus (Wilson and Nicoll, 2001) and in LTP induction (Carlson et al., 2002).

1.1.4(f) Histamine

Emerging evidences have shown that brain histamine plays a role in the modulation of memory consolidation via histamine type 1 (H1), type 2 (H2) and type 3 (H3) receptors (Serafim et al., 2012; Kohler et al., 2011; De Almeida and Izquierdo, 1986; 1988). Recent study showed that post-training histamine and histamine H_3 receptor antagonist thioperamide in the basolateral amygdala enhanced and impaired memory consolidation of the step-down inhibitory avoidance task, respectively (Benetti and Izquierdo, 2013). On the contrary, another study reported that systemic administration of H3 receptor antagonist, thioperamide facilitated memory consolidation with no influence on retrieval (Orsetti et al., 2001). In addition, a study by Cangioli et al. (2002) has shown that activation of H3 receptors by R-α-methyl-histamine in the basolateral amygdala improved the consolidation of the fear memory and enhanced the release of ACh.

1.1.4(g) Others

Glucose has been known to influence memory modulation (Izquierdo et al., 2004; Smith et al., 2011). Extracellular fluid of glucose in the brain was found to be low and even fluctuated across brain areas during spontaneous alternation testing

(McNay et al., 2001). In another study, glucose enhanced, and glucose-uptake inhibitor 2-deoxyglucose impaired memory consolidation in a day-old chick inhibitory avoidance model (Gibbs and Summers, 2002). Systemic or intra-amygdala administration of glucose led to the facilitation of the onset of extinction of drug-induced conditioned reward (Schroeder and Packard, 2003).

Another factor that may modulate learning and memory is bombesin. It was found that intrahippocampal infusion of RC-3095, the bombesin/gastrin-releasing peptide antagonist caused an impairment in the inhibitory avoidance in rats, after given immediately or two hours after training. This finding may suggest that the receptors for bombesin are present in the hippocampus, thus being able to modulate the memory consolidation (Roesler et al., 2003).

1.1.5 Memory circuitry in brain: interaction between neural systems

Based on the lesion of different brain areas, several neural systems have been shown to play a significant role in the processing information of different types of learning and memory (Gold, 2004; White and McDonald, 2002). Critical interactions between these neural systems will determine the time it takes to learn a task, and to the extent of the content and the strength of particular memories. Lesion in a particular area of the brain leads to a decreased contribution of a neural system to a memory processing from its normal proportional participation to a value of zero. However, if the brain damage led to enhancement of learning, it could be suggested that the neural system that contributes to the efficient learning is ‘released’ from its competition by a system that contributes to a less efficient learning (Gold, 2004). However, the degree of efficiency of the system varies between tasks.

As the memory research keeps developing, most of them have been concerned with the role of hippocampal formation in the formation and consolidation of the explicit memory (Wang and Morris, 2010; Richter-Levin and Akirav, 2000; Richter-Levin, 2004; Thierry et al., 2000). The hippocampal formation consists of the entorhinal cortex, dentate gyrus, the individual CA fields of the hippocampus proper and the subicular complex. Hippocampal formation works together with the subcortical and cortical networks where it is believed to store the long-term memory (Bird and Burgess, 2008; Wang and Morris, 2010; White and McDonald, 2002; Figure 1.5).

Prefrontal cortex is mainly implicated in the working memory as well as in temporal ordering of spatial and non-spatial events and in organization and planning of response. Both hippocampal formation and prefrontal cortex are anatomically and functionally associated and due to their reciprocal connections with the temporal and parietal association areas of the cerebral cortex, prefrontal cortex may serve as an intermediate link between these neocortical areas and the hippocampus (Thierry et al., 2000). Direct projection from the hippocampal formation to the prefrontal cortex was first described in 1977, in which projections from subiculum to the medial frontal cortex were found (Rosene and Van Hoesen, 1977). There were also reciprocal connections between caudal portion of the presubiculum and the dorsolateral prefrontal cortex (Goldman-Rakic et al., 1984). Direct projections from the hippocampal CA1 field to the medial and orbito prefrontal cortex were also observed (Barbas and Blatt, 1995).

Both prefrontal cortex and hippocampus further innervate the ventral striatum, particularly the nucleus accumbens which consists of two main regions, the ‘core’ and the ‘shell’. The ‘core’ is innervated preferentially by pre-limbic/medial

orbito areas of the prefrontal cortex while the 'shell' is innervated by the CA1/subiculum regions of the hippocampus. The nucleus accumbens plays a pivotal role in integrating information from the limbic system and cortical regions into goal-directed behaviours. Nucleus accumbens sends direct projections into several regions including pallidum, substantia nigra, hypothalamus and several mesencephalic areas (Thierry et al., 2000; Mogenson et al., 1982).

The amygdala is one of the brain regions that has a significant contribution to learning and memory processes. Neurons from the basal nucleus project to the entorhinal cortex, CA3 and CA1 fields of the hippocampus, subiculum and parasubiculum. These anatomical interactions are believed to be involved in the encoding and consolidation (Pare, 2003; McGaugh et al., 1996; Richter-Levin and Akirav, 2000), but not in the retrieval or expression of the emotionally based experiences, suggesting that amygdala only facilitates memory storage in other brain areas (Pare, 2003). It has been shown that amygdala activity modulated the induction of the hippocampal LTP (Akirav and Richter-Levin, 1999; 2002). Other than the hippocampus, the amygdala also receives extensive projections from cortical areas of the temporal lobe, from the substantia nigra, ventral tegmental area, nucleus accumbens and from brain stem areas (White and McDonald, 2002). In general, it can be concluded that one or more brain systems can be involved in acquisition, consolidation or retrieval processes of one particular types of memory.

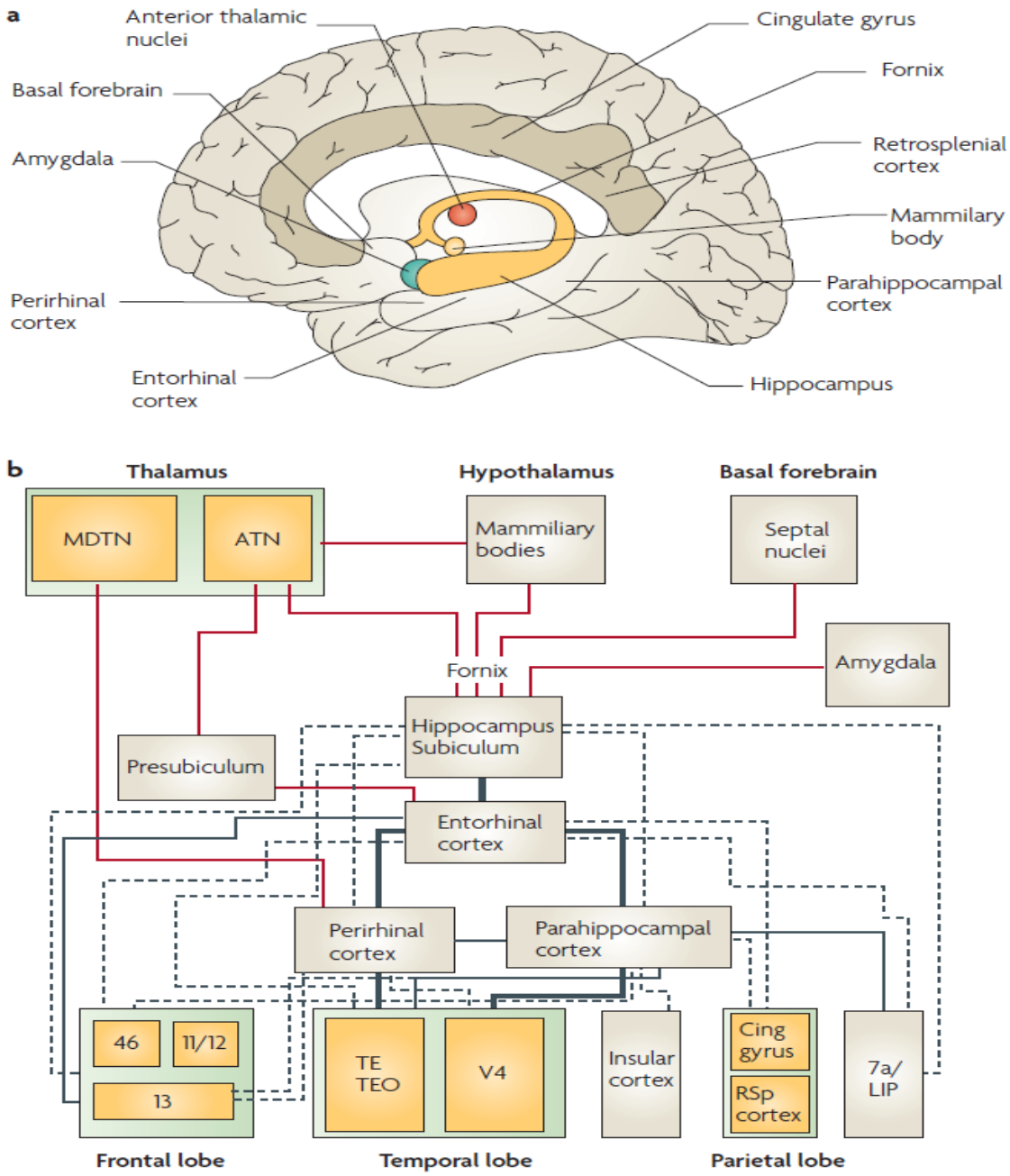

Figure 1.5: The hippocampus and its connections. a) Hippocampus in the medial temporal lobes b) Subcortical connections: red lines; cortical connections: black lines; thickness of the black lines approximates to the strength of the connections. Abbreviations: MDTN, medial dorsal thalamic nuclei; ATN, anterior thalamic nuclei; TE & TEO, inferior temporal areas TE and TEO; LIP, lateral intraparietal area; RSp, retrosplenial; Cing gyrus, cingulate gyrus (Adapted from Bird and Burgess, 2008).

1.2 Electroencephalogram (EEG)

EEG can be referred as amplified electrical activity generated by connecting neurons in the brain (Liu et al., 2005; Idris et al., 2014), thus is a reflection of the functional connectivity in the brain network. It has been proven to be a very sensitive and reliable tool for characterizing drug effects in the CNS (Palenicek et al., 2013; Dimpfel, 2005). It can be classified into delta (0.1-4 Hz), theta (4-7 Hz), alpha (7-13 Hz), beta (13-30 Hz) and gamma (>30 Hz) bands based on range of frequencies (Idris et al., 2014). Amplitude of brain waves is measured using microvolt unit (μV). Amplitude with less than 20 μV, in between 20-50 μV and more than 50 μV are considered as low, medium and high amplitude, respectively (Idris et al., 2014).

The slow wave delta band (0.1-4 Hz, 20-200 μV) is associated with deep sleep and unconsciousness while theta band (4-7 Hz, 20-100 μV) which originated from thalamus appears as consciousness slips towards drowsiness. The most prominent rhythm in the brain activity is alpha band (7-13 Hz, 20-60 μV), with round, sinusoidal or sometimes as sharp wave appearance. Alpha band appears mainly in the posterior half of the head, over the parieto-occipital region of the brain. This band indicates a relaxed awareness without any attention or concentration. Beta band (13-30 Hz, 2-20 μV) which usually found at frontal and central region represents the waking rhythm of the brain associated with active thinking or active attention. Lastly, the gamma band (> 30 Hz, ~5-10 μV) occurs at certain occasion when the sensory stimuli are given. EEG activity originated mainly from pyramidal postsynaptic potentials which are in oscillations with several brain regions. The first network is with thalamus, thus is referred as thalamocortical networks which is modulated by the reticulo-thalamo-cortical circuits. The other network is the extra-thalamic-cortical circuits which involve the reticular system, hypothalamus,

hippocampus, amygdala and basal forebrain nuclei. Finally, the network from the other cortex is referred as cortical-cortical networks (Idris et al., 2014).

EEG characteristics across species are relatively similar (Moretti et al., 2013; Sambeth et al., 2007). Changes in EEG characteristics induced by drugs were found to be similar in human and rodents (Dimpfel, 2005; van Luijtelaar et al., 2002; Dimpfel et al., 1992). These changes are useful in understanding the central activity of drugs in human (Sambeth et al., 2007). The electrical power of each frequency range changes in parallel with changes in particular behaviour or drug condition without affecting the neighbouring frequencies. This notion has been proven true by Stahl et al. (1997). Repeated exposure to d-amphetamine produced an increase in the alpha activity via activation of the dopamine D_2-like receptors, without affecting other frequency bands. Finding by Dimpfel and Schober (2001) further supported the notion. Administration of α_2-adrenoceptor agonist that acts presynaptically led to a great increase in electrical theta activity but not other frequencies. However, the degree of increment was not similar between brain areas suggesting uneven distribution of α_2-adrenoceptor in different brain areas.

Considerable evidences support the role of cholinergic system in modulating the brain activity (Dringenberg et al., 2002; Metherate et al., 1992). Blockade of stimulation-induced EEG activation by intracortical application of anti-muscarinic drugs suggested that acetylcholine promotes activation by a direct effect on cortical neurons (Metherate et al., 1992). In another study, cholinergic transmitter was found to modulate the changes in electrical delta activity which was intimately linked to dopaminergic transmission as shown by the concomitant changes in alpha2 electrical power (Dimpfel, 2005). The influence of the dopaminergic system on EEG activity was further corroborated via administration of dopamine agonist and antagonist,