NEUROPROTECTIVE EFFECTS OF TUALANG HONEY IN MALE RATS EXPOSED TO NORMOBARIC HYPOXIA

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by

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LIST OF ABBREVIATIONS

Ach	Acetylcholine
ACTH	Adrenocorticotrophic hormone
AMS	Acute motion sickness
AMPA	Aminomethylphosphonic acid
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BBB	Blood brain barrier
BDNF	Brain derived neurotrophic factor
BP	Barometric pressure
˚C	Celsius
CA	Cornu ammonis
CAT	Catalase
CGRP	Calcitonin gene related peptide
Cu	Copper
CVR	Cerebral vascular reactivity
DG	Dentate gyrus
dH_2O	Distilled water
DPPH	2,2-diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
FAMA	Federal Agricultural Marketing Authorities

Fe^{2+}	Ferrous cation
Fe^{3+}	Ferric cation
FO_2	Oxygen fraction
Ft	Feet
GAS	General adaptation syndrome
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
HACE	High altitude cerebral oedema
HH	Hypobaric hypoxia
HNE	Hydroxy-2-nonenal
H_2O_2	Hydrogen peroxide
HOONO	Peroxynitrous acid
HPA	Hypothalamic-pituitary adrenal axis
HWM	Hyper intense white matter
IL-8	Interleukin-8
i.p.	Intraperitoneal
i.v.	Intravenous
Kg	Kilogram
kGy	Kilogray
LPS	Lipopolysaccharide
LTM	Long term memory
M	Meter
MDA	Malondialdehyde

Mg	Milligram
µL	Microlitre
µM	Micromolar
MRI	Magnetic resonance imaging
Ng	Nanogram
NADPH	Nicotinamide adenine dinucleotide phosphate
NDCS	Neurologic decompression sickness
NF_kB	Nuclear factor kB
NH	Normobaric hypoxia
NMDA	N-methyl-D-aspartate
•NO	Nitric oxide anion
NORT	Novel object recognition task
NOS	Nitric oxide synthase
eNOS	Endothelial nitric oxide synthase
iNOS	Inducible nitric oxide synthase
nNOS	Neural nitric oxide synthase
O_2^-	Superoxide anion
OD	Optical density
OH	Hydroxyl radical
$ONOO^-$	Peroxynitrites
PBS	Phosphate-buffered saline
PO_2	Partial pressure of oxygen
PS	Power and sample size
RNS	Reactive nitrogen species
ROC	Receiver operating characteristic

ROH	Peroxyl radical
ROS	Reactive oxygen species
SEM	Standard error of mean
SOD	Superoxide dismutase
SPSS	Statistical Package for Social Science
STM	Short term memory
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TNF	Tumour necrosis factor
U	Unit
U/mL	Unit/millilitre
VEGF	Vascular endothelial growth factor
USA	United State of America
XO	Xanthine oxidase
Γ	Gamma
Zn	Zinc

KESAN NEUROPROTEKTIF MADU TUALANG DALAM TIKUS JANTAN TERDEDAH KEPADA HIPOKSIA NORMOBARIK

ABSTRAK

Pendedahan kepada persekitaran hipoksia boleh menyebabkan gangguan memori dan tekanan oksidatif dianggap sebagai salah satu mekanisme asas. Potensi produk semulajadi dengan sifat antioksidan untuk meningkatkan fungsi kognitif telah menjadi fokus banyak penyelidikan pada masa kini. Oleh itu, kajian ini bertujuan untuk menilai kesan hipoksia normobarik terhadap prestasi memori dan menjelaskan kemungkinan mekanisme sandaran madu Tualang dalam membaikpulih kemerosotan memori akibat hipoksia dalam tikus jantan dewasa. Empat puluh lapan ekor tikus Sprague-Dawley jantan telah dibahagikan kepada empat kumpulan: 1) tikus tidak terdedah hipoksia dirawat dengan sukrosa, 2) tikus tidak terdedah hipoksia dirawat dengan madu, 3) tikus terdedah hipoksia dirawat dengan sukrosa, 4) tikus terdedah hipoksia dirawat dengan madu. Madu diberikan secara oral, 0.2 g/kg selama 14 hari sebelum terdedah kepada hipoksia. Semua haiwan eksperimen diuji dengan ujian pengenalan objek asing (NORT) dan T-maze sebanyak dua kali iaitu sebelum rawatan madu dan selepas terdedah kepada hipoksia. Tikus didedahkan kepada hipoksia dengan menempatkannya dalam kebuk hipoksa pada kandungan O_2 ~ 11%. Apabila selesai menjalankan eksperimen tikus dibunuh. Sampel darah dan otak diambil. Hemispheres kanan otak telah disimpan dalam formalin 10% untuk kajian histologi rantau hipokampus. Hemisfera kiri otak dihomogenasi, diempar dan digunakan untuk analisa tahap/aktiviti katalase (CAT), glutation peroksida (GPx), superoksida dismutase, kapasiti antioksidan total (TAC) dan malondialdehid (MDA)

menggunakan teknik enzim-esei imunoserapan berkaitan (ELISA). Serum kortikosteron diukur menggunakan kit ELISA yang tersedia secara komersil. Penemuan daripada kajian ini menunjukkan penurunan prestasi memori, bilangan sel positif Nissl di semua rantau hippocampus dan tahap/aktiviti enzim antioksidan otak, dan peningkatan tahap MDA otak dan kortikosteron serum dalam kumpulan tikus terdedah hipoksia berbanding kumpulan eksperimen lain ($p<0.05$). Peningkatan prestasi memori dan tahap/aktiviti TAC, CAT, GPx dan SOD otak, pengurangan tahap MDA otak dan kortikosteron serum serta penghalangan apoptosis sel neuron di semua rantau hippocampus dalam tikus terdedah hipoksia yang dirawat dengan madu berbanding sukrosa ($p<0.05$). Kesimpulannya, pra-rawatan dengan madu Tualang pada dos 0.2 g/kg selama 14 hari berpotensi untuk digunakan sebagai terapi alternatif untuk mencegah kemerosotan prestasi memori akibat terdedah hipoksia.

NEUROPROTECTIVE EFFECTS OF TUALANG HONEY IN MALE RATS EXPOSED TO NORMOBARIC HYPOXIA

ABSTRACT

Exposure to environmental hypoxia can induce memory impairment and oxidative stress is thought to be one of the underlying mechanisms. The potential effects of natural products with antioxidant properties to enhance cognitive function have been the focus of many researches nowadays. Thus, the present study aims to evaluate the effect of normobaric hypoxia on memory performance and elucidate the potential mechanisms of Tualang honey in improving hypoxia-induced memory impairment in adult male rats. Forty-eight male Sprague-Dawley rats were divided into four groups: 1) sucrose treated non-hypoxia, 2) honey treated non-hypoxia, 3) sucrose treated hypoxia, 4) honey treated hypoxia. The honey was administered orally, 0.2 g/kg for 14 days before exposure to hypoxia. All experimental animals were subjected to novel object recognition task (NORT) and T-maze twice i.e. prior to honey treatment and after exposure to hypoxia. Rats were exposed to hypoxia by placing them in a hypoxic chamber at O_2 content of ~ 11%. After completion of the experiment, the rats were sacrificed. Their blood and brain samples were collected. The right brain hemispheres were fixed 10% formalin for histological study of the hippocampal region. The left brain hemispheres were homogenised, centrifuged and used for analysis of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), total antioxidant capacity (TAC) and malondialdehyde (MDA) levels/activities using enzyme-linked immunosorbent assay (ELISA) technique. Serum corticosterone was measured using commercially available ELISA kits. The findings from the present study showed significant effects of hypoxia exposure on

impairment of memory performance, decrease in number of pyramidal cells in all hippocampal regions, decrease in levels and activities of antioxidant enzymes and increase in MDA in the brain homogenate, and serum corticosterone level in sucrose treated hypoxia group compared to other experimental groups (P<0.05). Improved memory performance, increased levels/activities of brain TAC, CAT, GPx and SOD, reduced brain MDA and serum corticosterone levels, prevented neuronal cells apoptosis in all hippocampal regions in honey treated hypoxia group compared to sucrose treated hypoxia group (P<0.05). In conclusion, Tualang honey pre-treatment at a dose of 0.2 g/kg for 14 days has the potential to be used as an alternative therapy to protect against hypoxia-induced memory impairment.

CHAPTER ONE

INTRODUCTION

1.1 Memory

1.1.1 Definition

Memory is defined psychologically as the process in which the information is encoded (initial acquisition of memory), stored (reservation of memory for a long time) and retrieved (reactivation of previously stored memory) (Cohen and Stackman Jr, 2015). Cognitive neuroscientists have considered memory as the retention, reactivation and reconstruction of the experience-independent internal representation (Dudai, 2004). Others have guessed that the memory does not exist until it is revealed in behaviour and thought (Talmi et al., 2007).

Memory and learning are considered as a family of distinct processes, each with its own role, not a single process. Memory is the process of knowledge retention, and learning is the process of information acquiring (Bailey et al., 1996).

1.1.2 Models

Atkinson and Shiffrin (1968) have suggested the information is transferred from short-term memory (STM) to long-term memory (LTM) after a short period of time, the most popular model on studying the memory known as the multi-store model or Atkinson model. Both STM and LTM can be weakened due to age, diseases or a variety of other reasons that affect the memory.

The LTM can be further classified into declarative (explicit) and non-declarative memory (implicit). Declarative (explicit) memory is defined as the ability to remember the facts, events and personal history, required conscious thought and dependent on the interconnected structures in the medial temporal lobe. Non-declarative (implicit) memory does not require conscious thought. Declarative memory has been mostly studied in mammals, whereas non-declarative memory has been effectively studied in both non-mammalian vertebrates and higher invertebrates (Bailey et al., 1996). Figure 1.1 shows the classification of memory.

1.1.2 (a) Short-term memory

The STM is referred to as "the brain's post-it note". It can be defined as the ability to remember and process the information. A "magical number seven, plus or minus two" is considered the most seminal paper in the literature for evaluation of limits in STM storage capacity (Miller, 1956), however modern estimates of STM capacity are lower, typically of about 4 to 5 items (Cowan, 2001) and this information requires conscious effort quickly to retain it. The STM relied mostly on acoustic code and to a lesser extent on visual code (Conrad and Hull, 1964).

1.1.2 (b) Long-term memory

The LTM stores larger quantities of information for longer time semantically, sometimes the whole life span (Baddeley, 1966). The information is stored in two forms verbal code and non-verbal code (typically visual). After consolidation, LTM is persistent and stable.

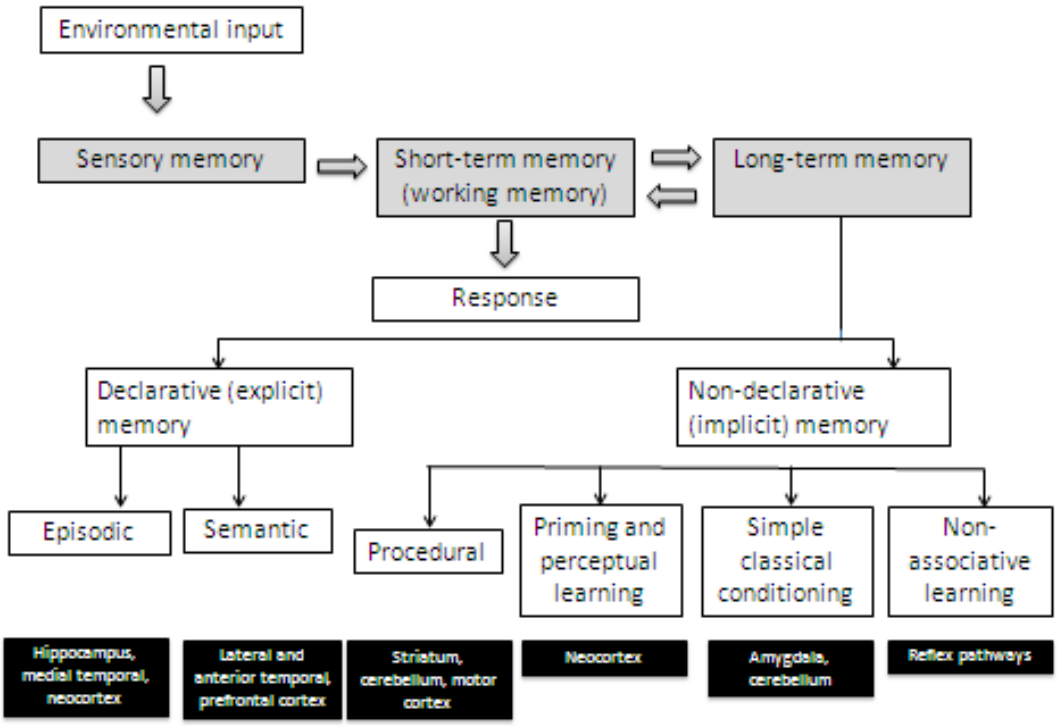

Figure 1.1 Classification of memory and brain regions involved (Adapted from Bartsch and Butler, 2013)

The LTM is maintained by permanent physical changes in the structure of neurons widely spread throughout the brain, whereas STM is supported by transient pattern of neuronal communication in frontal and parietal lobes of the brain. The transfer of information from STM to LTM is facilitated by mental repetition of information, motivation or more effectively by associating it with other previously acquired knowledge and giving it a meaning (Bailey et al., 2004).

1.1.3 Memory consolidation

Consolidation is the process in which the hippocampus continually activates the neocortex to stabilize the STM after its initial acquisition, to be converted to LTM (Dudai, 2004). Consolidation can also be defined as a labile phase during which the memory trace will be stored physically (Quillfeldt, 2010). The consolidation of STM into LTM occurs at the molecular level in neurons and involves the new proteins synthesis that regulates neuronal morphology and connectivity (Bekinschtein et al., 2008).

A growing body of evidence shows that brain-derived neurotrophic factor (BDNF) plays an important role during memory formation by the regulation of both short-term synaptic function and long-term synaptic plasticity (Bekinschtein et al., 2007). Furthermore, it has been found an increase in expression of BDNF in the young rat hippocampus during exercise is associated with subsequent memory enhancement (Garza et al., 2004; Berchtold et al., 2010). Consolidation involves three processes: synaptic consolidation, systemic consolidation and reconsolidation.

1.1.3 (a) Synaptic consolidation

The standard model of synaptic consolidation postulated that changes in the protein synthesis process and in membrane potential in the medial temporal lobe occurs through activating intracellular transduction cascades (Dudai, 2004; Bramham and Messaoudi, 2005). These molecular cascades activate transcription factors that cause changes in gene expression. Gene expressions lead to alteration in synaptic proteins and synaptic remodelling and growth (Dudai, 2004). The process of synaptic consolidation takes place within minutes to hours of learning and memory encoding. After six hours of training, memories can resist any interference that disrupts synaptic consolidation and subsequent LTM formation (Dudai, 2004).

1.1.3 (b) Systemic consolidation

Systemic consolidation is considered as reorganization process during which the transfer of the hippocampus-dependent memory into hippocampus-independent memory that lasts months to years (Alvarez and Squire, 1994). The process of systemic consolidation takes place within six hours of learning and information encoding. Later on, the representation of this information in the hippocampus becomes active temporarily (Squire and Alvarez, 1995). During this period, the hippocampus transfers the information into the cortex making the memory becomes hippocampal-independent and lasts for a long time (Squire and Alvarez, 1995; Dudai, 2004). As a result of its self-generated network patterns, the process of memory consolidation allows the newly acquired memories to be gradually transferred to neocortical stores where it becomes permanently stored.

The hippocampus, as the brain's search engine also permits a fast and efficient search among the deposited memories in the neocortex, which is a process necessary for planning the future and generating creative ideas. The hippocampus receives input from different parts of the cortex and sends output to different parts of the brain (Squire and Alvarez, 1995).

1.1.3 (c) Reconsolidation

Reconsolidation is recently considered as a focus of research and can be defined as a process in which the previously consolidated memory becomes labile again through reactivation of memory trace. Memory retrieval is not a passive phenomenon. It activates a reconsolidation (second memory consolidation cascade) through triggering a number of processes that either reinforces or alters stored information that requires protein synthesis. Memory reconsolidation is dependent on the strength and duration of the memory, such that the shorter and weaker memories are more easily reconsolidated than the longer and stronger memories (Suzuki et al., 2004).

1.1.4 Role of hippocampus in different types of memory

There are two hippocampi, one in each side of the brain. It is a part of the limbic system and located under the cerebral cortex in humans and in the medial temporal lobe, underneath the cortical surface in primates. In the rat, the two hippocampi are connected at the stems by the hippocampal commissure that crosses the midline under the anterior corpus callosum (Figure 1.2). In human and monkey, the part of the hippocampus at the top is much narrower than the part at the bottom.

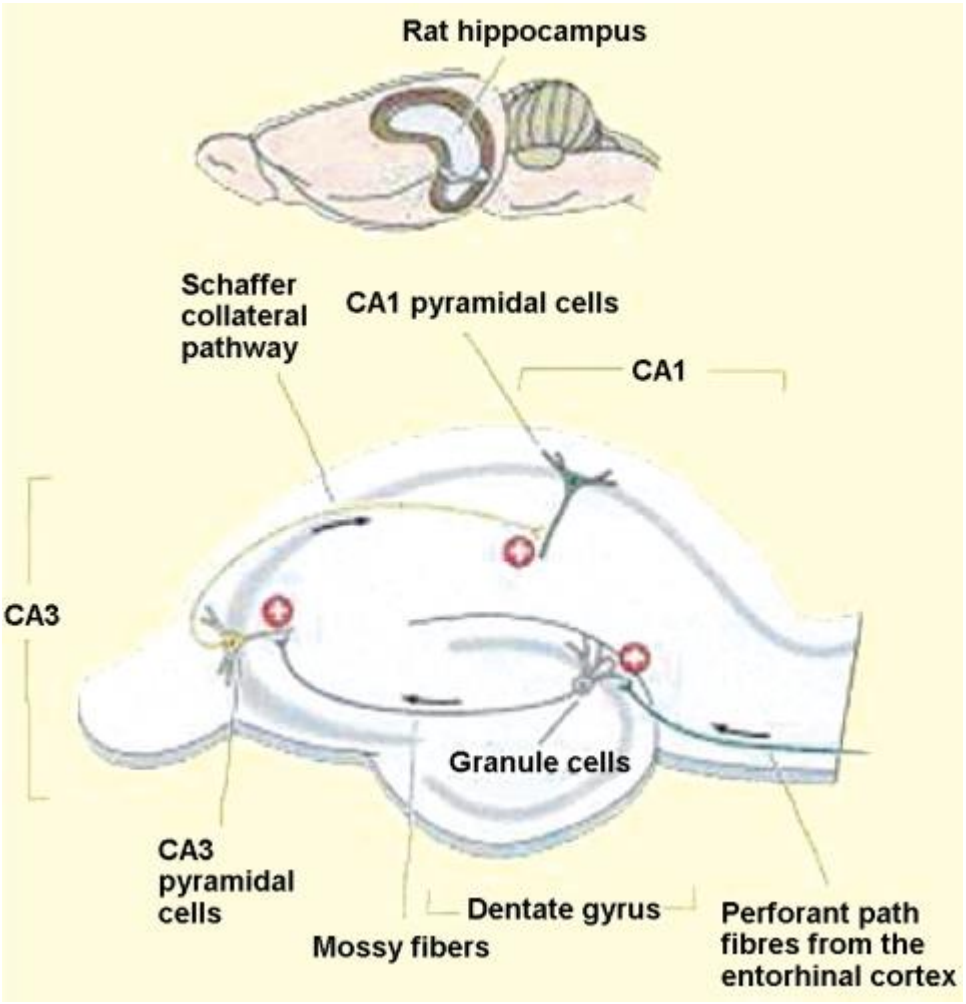

Figure 1.2 Anatomy and histology of rat's hippocampus (Adapted from Neuroscience, 2012)

The cross-sections through the hippocampus show different shapes, depending on the location and angle of the cut because of its complex geometry (Tulving and Markowitsch, 1998).

Researches on humans and experimental animals have concluded that most anatomical details and functional role of the hippocampus and adjacent cortical areas are conserved across species (Manns and Eichenbaum, 2006). Since several neuronal cell types are uniformly organized into layers in the hippocampus, it has frequently been used as a model system for neurophysiologic study. Long-term potentiation (LTP), a form of neuronal plasticity, was first discovered to occur in the hippocampus and had often been studied in this structure (Bliss and Collingridge, 1993).

The cortico-hippocampal circuit for declarative memory consists of three components: the cortex, the para-hippocampal region and the hippocampus itself. The beginning and the endpoints of this circuit includes several association cortical areas whose outputs converge on the para-hippocampal area. The para-hippocampal region consists of interconnected perirhinal, postrhinal and entorhinal cortices that surround and send the cortical input to the hippocampus: the main function of the hippocampus is to combine the incoming information about spatial and non-spatial items from the cortex (Manns and Eichenbaum, 2006). Within the hippocampus, there are several stages of serial processing occur and the outcome of this integration is relayed back to the para-hippocampal region which then sends the main output to the same cortical areas that provided the input (Eichenbaum, 1997).

1.1.4 (a) Recognition memory

Recognition memory is the most widely studied subtype of declarative memory and defined as the ability to recognise the previously encountered objects, events or people (Wais et al., 2006). The elicited matching signal will be activated when the previously experienced event has been re-experienced again (Norman and O'Reilly, 2003). Recognition memory retrieval involves two processes: familiarity or semantic and recollection or episodic. Familiarity is the immediate automatic feeling that the event, individual or item was previously experienced. Recollection is the slower process during which full attention to the present stimuli stimulates conscious recall of contextual details of the previously encountered event (Cohen and Stackman Jr, 2015).

The anatomical basis of this two distinct retrieval process of recognition memory is controversial. Previous studies have shown that both familiarity and recollected retrieval process of recognition memory are functionally independent and relied on a distinct neuronal network (Yonelinas et al., 2005; Curran et al., 2006). Previous human imaging studies have revealed activation pattern of region-specific areas of the brain associated with recollection and familiarity process of recognition memory. Another study has proposed the cortical areas along the para-hippocampal gyrus are involved in recognition memory and this evidenced by findings that have shown that good recognition performance was observed following restricted hippocampal lesions in case of developmental amnesia and in a patient with adult-onset amnesia (Mayes et al., 2002) These finding strongly support the view that suggested the recollective process relies on hippocampus while familiarity component is supported by cortical areas in para-hippocampal region (Yonelinas et al., 2005; Curran et al.,

2006). Another study has suggested both recollection and familiarity processes are supported by the hippocampus (Wais et al., 2006).

The previous study on rats has used adapted receiver operating characteristic (ROC) curve to examine odour recognition memory and the results have shown that the hippocampus mediates the recollected process of recognition memory. In humans; there are limited numbers of methods that can reliably separate judgement based on recollection from judgement based on familiarity. Numerous functional imaging studies on amnestic humans have shown that separate region-specific neural activation patterns are associated with recollection and familiarity processes, this strongly supports that familiarity and recollection process are supported by the different underlying system (Cohen and Stackman Jr, 2015).

Recognition tasks have been done in animals models and the right hippocampus is specifically for place recognition (spatial memory). The object recognition task is used mainly to investigate recognition memory, STM and LTM. Dual-process models of recognition memory in animals, using novel object recognition task, suggest that recognition memory is supported by involved in memory tasks that require allocentric processing of spatial location (Burgess et al., 2002). It can be classified into two major paradigms: object recognition and place recognition. Different brain structures have functioned separately and this has been shown by two independent processes that are the context-dependent process (hippocampal-independent) and familiarity process (hippocampal dependent).

Quillfeldt (2016) reported that healthy rats preferred to explore a novel object more than familiar one (familiarity component). They have also preferred to explore familiar object presented at many spatiotemporal contexts more than familiar objects presented either in different temporal or spatial contexts (episodic component). Rats with hippocampal lesions showed intact familiarity component and impaired context-dependent components of recognition (Good et al., 2007). The T-maze is used to distinguish the motor activity and spatial learning. Also, it can test different types of memories: LTM, working memory and egocentric memory (Quillfeldt, 2010).

1.1.4 (b) Spatial recognition (spatial memory)

Spatial navigation theory in an animal has postulated that the hippocampus of rats and other animals provide the basis for spatial memory and navigation (Burgess et al., 2002). Place cells are neurons in the hippocampus of rat and mouse where their response represented as an elaboration of the action potential when the animals expose to specific part its location and environment. This, however, does not appear to reflect a species difference between animals and humans. Instead, it has been found that neural algorithms are the same as those used for the navigation in cognitive space during recall and planning (Maguire et al., 1999).

The cognitive map theory postulated that the mental representation of allocentric spaces is created by the hippocampus, has the ability to create novel routes between familiar locations, and more flexible compared to other mental representations of space (Morris et al., 1982). In humans, cognitive map theory has stated that the hippocampus has a broader function based on lateralization of function whereby

spatial memory appears to be confined more specifically to the right hippocampus (Burgess et al., 2002).

The parallel map theory postulates that the two major parts of and the hippocampus proper (HP), play different roles in spatial navigation. The parallel map theory proposed that the spatial navigation is created by hippocampus proper (HP) and the dentate gyrus (DG) parts of the hippocampal formation (HPF) (Jacobs and Schenk, 2003). The inherent dual nature of spatial navigation for both positional and directional landmarks is a result of the dual function of hippocampal regions. These landmarks can be modulated to form parallel spatial representations, both bearing map of DG and CA3 and sketch map of CA1. This theory states that the parallel map involves two independent and complementary maps: bearing map and sketch map, each contributes to creating an efficient and effective representation in assembly-line fashion, integral map (Jacobs and Schenk, 2003).

The parallel maps differ from the cognitive maps in used cues to represent space, how to represent space, and the hippocampal regions that create the representation. The parallel maps theory can be implicated profoundly for spatial navigation's principles and the hippocampal function in mammals (Jacobs and Schenk, 2003).

1.1.4 (c) Non-spatial recognition

The hippocampus is important in rodent non-spatial memory (Cohen and Stackman Jr, 2015). The previous study has shown that hippocampal lesion either temporary or permanent consistently impairs the recognition memory in object recognition task

after more than 10 min delay was imposed between the acquisition and test session (Cohen and Stackman Jr, 2015).

Several previous studies have shown that the structures within medial temporal lobe work independently as evidenced by impairment of spatial memory task in rats with hippocampal regions damage compared to control and those with cortical region damage rats (Winters et al., 2004). Impaired recognition of the familiar object/location and difficulty in distinguishing it from novel object/recognition, early trait of cognitive decline, was observed in patients with schizophrenia and Alzheimer's disease who develop impairment of declarative recognition memory which is supported by medial temporal lobe, hippocampus, peri-rhinal cortex, ento-rhinal cortex and para-hippocampal cortex (Squire et al., 2007).

1.2 Hypoxia

1.2.1 Definition

Hypoxia literally means low oxygen content of air detrimental to the aerobic organism. The decrease in oxygen content in the tissues is known as oxygen starvation whereas complete lack of oxygen supply in the tissue is known as anoxia (Bickler and Buck, 1998).

1.2.2 Classification

Hypoxia can be classified according to the region of the body affected into generalized (affected the whole body) and localized (affected area of the body). It can be also classified according to the duration of hypoxic exposure into acute (from

minutes to hours), chronic (from hours to many years) and life-long hypoxia (high altitude residents) (Møller et al., 2008).

Hypoxia can be classified into hypoxic hypoxia, affinity hypoxia (due to failure of haemoglobin to release the oxygen to the tissue), anaemic hypoxia (results from reduction of the oxygen-carrying capacity of the blood), histotoxic hypoxia (as a result of decreased utilization of oxygen by the tissue as in cyanide poisoning) and circulatory hypoxia (due to insufficient blood flow as in heart failure). Hypoxic hypoxia can be sub-classified into hypobaric hypoxia and normobaric hypoxia, each can be induced by reduction of ambient partial pressure of O_2 (PO_2) either by lowering barometric pressure in hypobaric hypoxia (HH) or by decreasing the O_2 fraction in normobaric hypoxia (NH) (Qaid et al., 2017).

1.2.3 Physiological responses to hypoxia

The first stage of this response begins with activation of the sympatho-adrenal system leading to increased alertness, diversion of blood from internal organs to skeletal muscles, increased heart and respiratory rate and conversion of stored glycogen into glucose (Wong et al., 2010). In a case of more prolonged hypoxia exposure persists, the energy stores are depleted and hypothalamic-pituitary-adrenal axis (HPA) is stimulated and associated by an increase in production of adrenocorticotrophic hormone and corticosterone (Ursin and Eriksen, 2004). However, in a case of severe hypoxia, the pathological response to hypoxia is activated.

1.2.4 Molecular mechanisms of hypoxic cell damage

The molecular mechanisms of hypoxia cell damage were illustrated in Figure 1.3. During the period of hypoxia, progressive and uncontrollable axonal depolarisation of neurons occurs. Anoxic depolarisation is caused by impairment of Na^+/K^+ ATPase pump as a result of losing the energy required to maintain this ionic pump. It is characterised by increased in intracellular Na^+ and Ca^{2+} and increased in extracellular K^+, glutamate, aspartate and adenosine triphosphate (ATP) (Stys, 1998). Excess glutamate release into extracellular environment cause excitotoxicity which leads to uncontrollable opening of glutamate receptors involving N-methyl-D-aspartate (NMDA) and aminomethylphosphonic acid receptors (Rodrigo et al., 2005; Champagne et al., 2008). Activation of these receptors allows the outflow of ATP to the extracellular space which with excess glutamate will cause increased intracellular Ca^{2+} level (Godukhin et al., 2002; Weilinger et al., 2013).

Intracellular Ca^{2+} overload is the critical step in the development of neuronal damage via apoptosis and necrosis (Rodrigo et al., 2005). The Ca^{2+} overload leads to increased reactive nitrogen species (RNS) production especially nitric oxide (NO), up regulation of nitric oxide synthase (nNOS), increased reactive oxygen species (ROS) production, deactivation of superoxide dismutase (Okamoto et al.), catalase (CAT), and glutathione peroxidase (GPx), many organs dysfunction and activation of apoptotic enzymes and genes (Rodrigo et al., 2005).

The NO production leads to mitochondrial dysfunction which results in the release of superoxide and cytochrome-c from mitochondria into the cytosol. (NO-superoxide) interaction form peroxynitrite ($ONOO^-$), an oxidative molecule that causes oxidative

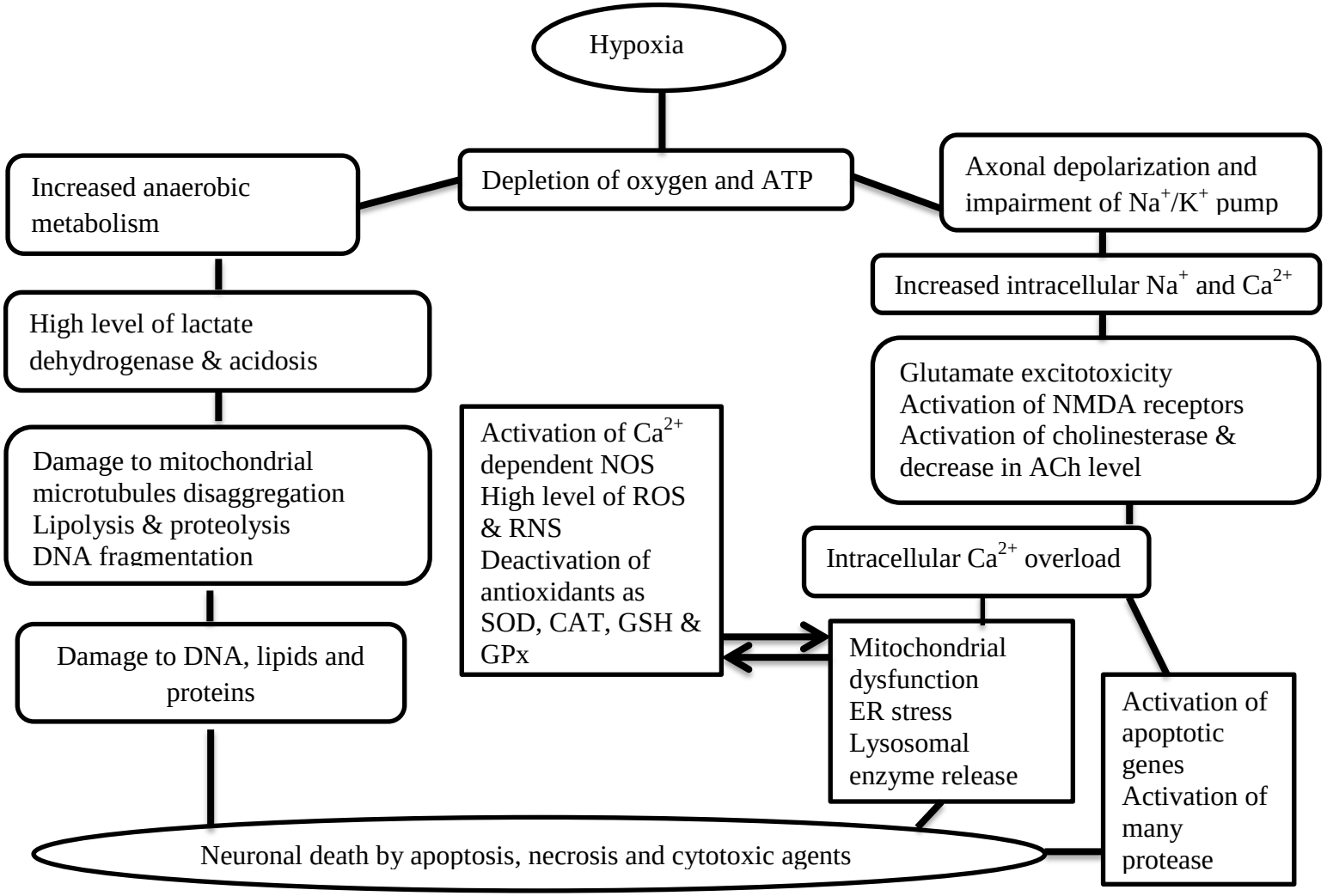

Figure 1.3 Mechanisms of cellular death during hypoxia

damage to DNA, lipids and proteins. Cytochrome-c release leads to activation of caspase-3 and deactivation of the antioxidant system include SOD, CAT and GPx (Rodrigo et al., 2005; Kritis et al., 2015).

Mitochondrial, endoplasmic reticulum (ER) and lysosomal dysfunction play a pivotal role in neuronal death. The consequences of mitochondrial dysfunction are upregulation of phospholipase-A2 and xanthine oxidase, increase in ROS production, deactivation of CAT, SOD and GPx and decreased ATP production (Jiang et al., 2011; Kritis et al., 2015). Depletion of ATP results in bioenergetics failure and neurodegeneration. Production of ROS causes mitochondrial and cellular DNA, protein and lipid damage (Jiang et al., 2011; Kritis et al., 2015). ER-stress via unfolded protein response (UPR) results in activation of proteasomes and chaperones that cause additional cellular damage (Kritis et al., 2015). Lysosomal dysfunction causes the release of hydrolytic enzymes as lipase, nuclease and protease which stimulate autophagy (Kritis et al., 2015). The release of enzymes as caspase-3 and calpains has been reported to play an essential role in mediating apoptosis during hypoxia (Zhang et al., 2006; Gao et al., 2014).

1.2.5 Cellular death during hypoxia

The results of all these factors discussed in 1.2.4 are synaptic dysfunction, neural plasticity impairment and cell death (necrosis, apoptosis and autophagy). Apoptotic and necrotic cell death depends on the severity of hypoxia, with acute hypoxia the neural death is necrotic in nature (proliferation of endoplasmic reticulum, disaggregation of polyribosome and dendritic swelling) with underlying mechanism is disturbance of ionic balance and increases in free radicals production, while with

chronic hypoxia the neural death is apoptotic in nature (chromatin and cytoplasmic condensation, membrane blebbing, DNA fragmentation and formation of apoptotic bodies) caused by activation of many apoptotic genes such as caspase (Maiti et al., 2008).

Another cause of cell death is due to lactate accumulation and acidosis. The consequential damage includes DNA fragmentations, cell swelling, lipolysis and proteolysis (Huang and Castillo, 2008).

1.2.6 Hypoxia in human

Many populations have inhabited high altitude regions, as a result of an ever-growing world population, i.e. about 140 million people live at an altitude above 8000 ft. (Moore et al., 1998) and about million population work in and travel to high altitude region (Leissner and Mahmood, 2009). Tourists, anaesthesiologists and mountaineers have enjoyed travelling to altitude 2500 – 8000 m for skiing, hiking and recreation (Sarkar et al., 2003; Leissner and Mahmood, 2009). The worldwide international transports were about 664 million in 2000, and also about 80% of increasing travel to a high altitude between 1995 and 2010 was predicted by the World Tourist Organisation (Zuckerman, 2002). Stressors of flight involve high rate of ascent, high level of altitude, low pressure, hypoxia, hypothermia, noise, radiation, vibration, and toxicological threats. Ernsting (1984) has mentioned that hypoxia is the most important single hazard at high altitude that cannot be avoided without sufficient oxygen supply. The partial pressure of oxygen decreases when the altitude increases as a result of lower barometric pressure (Moore et al., 1998). The causes of hypoxia

at high altitude are failure of oxygen delivery, carbon monoxide inhalation and cabin pressure failure.

1.2.7 Hypoxia and brain

Exposure to hypoxia induces a harmful effect on neurological functions (Leissner and Mahmood, 2009). Brain is the most susceptible organ to hypoxia for the following reasons: high content of polyunsaturated fatty acids, high metabolic rate that utilise high oxygen level and high level of hydroxyl radical formation due to high content of copper and iron transition metals and low antioxidants defence activity (Ames et al., 1993; Maiti et al., 2006; Cobb and Cole, 2015).

Hippocampus is the most vulnerable region of the brain to hypobaric hypoxia (Hota et al., 2007) due to large number of glutamate receptors that trigger the pathologic consequences during hypoxia (Shigemoto et al., 1997; Arora et al., 2017), high expression of immediate early gene and heat shock protein (Busl and Greer, 2010), deep blood supply and high levels of glucocorticoid receptors that affect the hippocampus in at least three ways: reduction of neuroexcitation in hippocampal regions, inhibition of neurogenesis and inducing dendritic atrophy in CA3 region of hippocampus (Champagne et al., 2008). The CA1 is more susceptible than CA3 to hypoxic cellular damage due to higher expression of P75 gene (Hota et al., 2007; Maiti et al., 2008); however, other studies showed that CA3 is more susceptible due to higher metabolic rate (Titus et al., 2007). The pyramidal neurons are more vulnerable than glial cells because they consume higher energy, conduct action potential and produce glutamate (Blomgren and Hagberg, 2006).

It has been found that lifestyle is considered an important factor that maintains neural function during individual's lifespan. The decrease in the partial oxygen pressure (PO_2) in a hypoxic chamber is generally used to study the effect of hypoxia in life sciences. This is performed either by lowering the oxygen fraction (FO_2) (normobaric hypoxia) as shown by (Barcroft, 1925) or by a decrease in barometric pressure (BP) (hypobaric hypoxia), without a change in FO_2, as shown by Bert (1878) in the late 1800s.

Many factors determine the severity of hypoxia effect on the human which include: altitude level, duration at altitude, rate of ascent to altitude, oxygen content of breathing gas, physical activity, temperature value, and individual response activity and their acclimatization to high altitude, and genetic factor (Yang et al., 2009; Simonson et al., 2010; Petrassi et al., 2012; Bogdanova et al., 2014). Hypoxia symptoms in most people generally begin to appear above 10 000 ft., people who are between 25 and 50 years old, healthy and active generally have a higher resistance. Symptoms in people who are stressed, ill or fatigued can often be noticed at an altitude lower than 8000 ft. It was found that there are some individual variability in acute motion sickness (AMS) symptoms which may be referred to some genetic predisposition to AMS, can be generalized individual response to hypobaric hypoxia, that seems to be polygenic and determined by different genes expressions encoding different proteins as hypoxia inducible factor-1, erythropoietin and nitric oxide synthase (Yang et al., 2009; Simonson et al., 2010; Carod-Artal, 2014). It has been found that some people acclimatized to high altitude while other people cannot (Yang et al., 2009; Simonson et al., 2010).

The symptoms of hypoxia were illustrated in Figure 1.4. Symptoms in mild to moderate hypoxia (less than 11000 ft.), an altitude sickness symptoms (acute motion sickness, high altitude cerebral oedema and high altitude pulmonary oedema), are light headache, fatigue, numbness/tingling of extremities, nausea and anorexia with no alteration in body weight (Bogdanova et al.,2014). The underlying mechanisms may refer to physiological response in vascular, respiratory, haematological systems to shift the blood to the brain as shown in Figure 1.5.

In severe hypoxia (up to 18000 ft.) or hypoxia with rapid onset, the symptoms include anorexia, weight loss, ataxia, confusion, hallucination, learning and memory impairment, behavioural changes, reduced level of consciousness, coma and death (Turner et al., 2015). This is coinciding with studies that showed that the cognitive function including learning and memory are affected at an altitude above 4500 m (15000 ft.) (Roach and Hackett, 2001; Bogdanova et al., 2014), and more profoundly impaired at an altitude more than 6000 m (Carod-Artal, 2014). Deterioration is another condition, characterised by anorexia, weight loss, fatigue, lethargy, and thought slowness with poor judgement, experienced at extreme altitude and reported to develop over weeks at altitude above 5500 m and over days at altitude above 8000 m (Milledge et al., 2007; Paralikar and Paralikar, 2010; Yaron and Honigman, 2014).

Earlier studies suggest that changes in dendritic morphology could contribute to both ‘progressive’ and ‘regressive’ plasticity in terms of learning and memory (Rao and Raju, 2012). Other studies have shown that oxidative stress and the subsequent hippocampal morphological changes are considered one of the underlying mechanisms (Maiti et al., 2008).

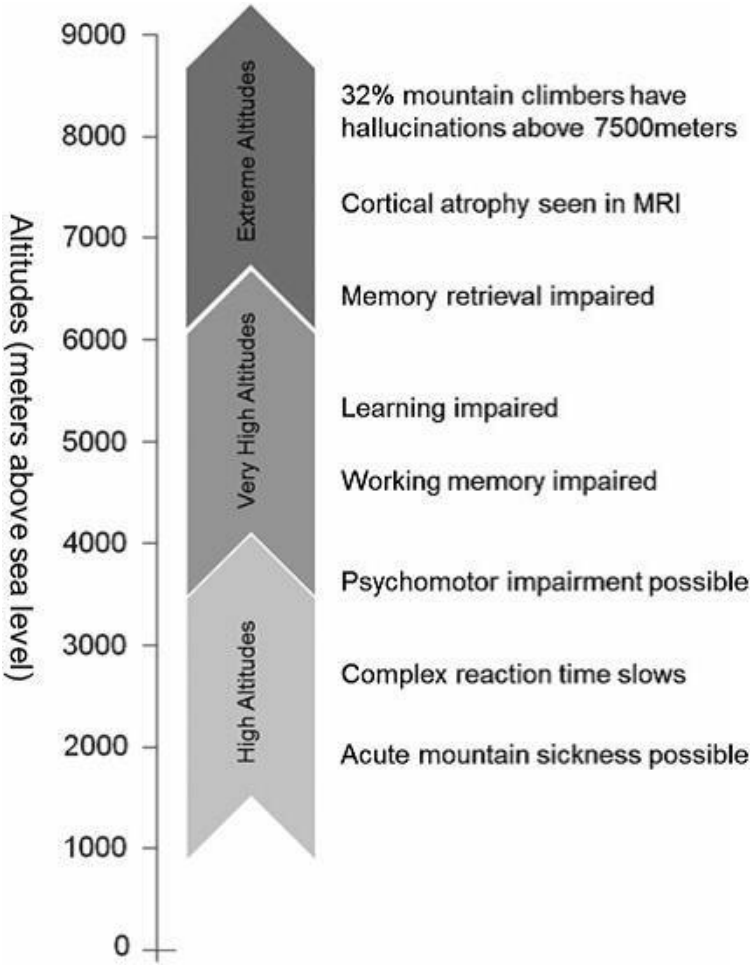

Figure 1.4 Hypoxia symptoms at different levels (Adapted from Wilson et al., 2009)

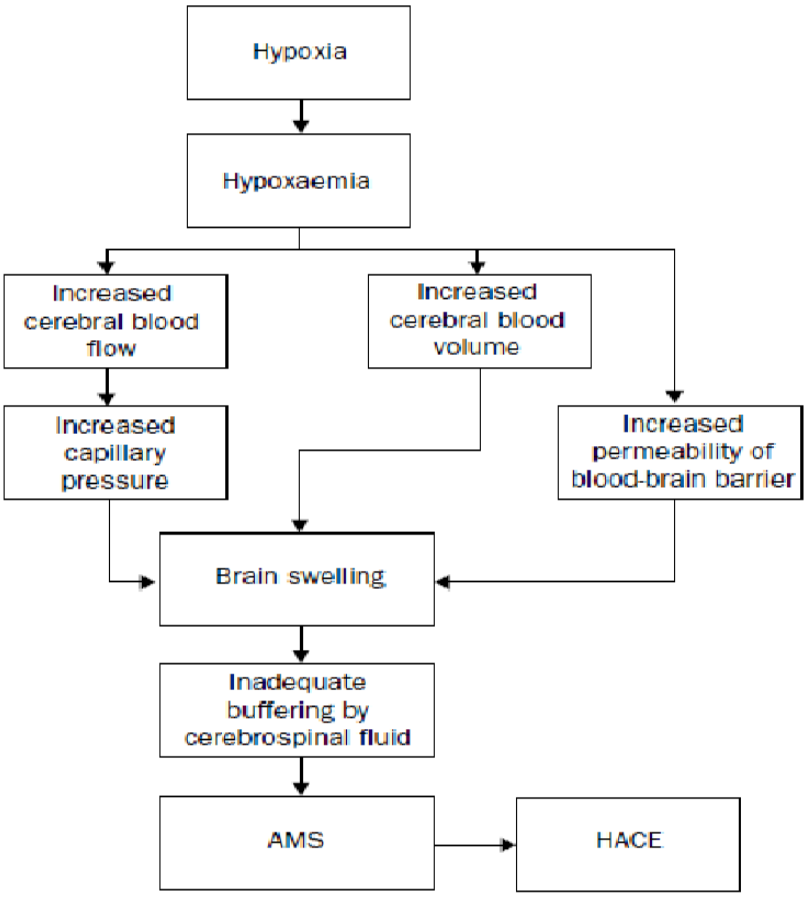

Figure 1.5 Hypoxia-induced cerebral oedema (Adapted from Basnyat and Murdoch, 2003)

Volunteer ascent to high altitude 5050 m for 3 weeks has shown loss of grey matter volume and improvement in cerebral vascular reactivity (CVR) (Foster et al., 2015). A previous study on 50 U-2 pilot volunteers has shown that 12 U-2 pilots have experienced clinical neurologic decompression sickness (NDCS) compared to 38 U-2 pilots who did not develop neurological impairment. The collected imaging data using a 3T magnetic resonance imaging scanner and high-resolution three-dimensional fluid-attenuated inversion recovery sequence has shown that there was a significant increase in hyper-intense white matter lesion (leukoaraiosis) in NDCS group due to hypobaric hypoxia (McGuire et al., 2012). Acute exposure to high-altitude has been associated with impairment of short-term memory and cognitive dysfunction (Virues-Ortega et al., 2004); this coincided with another study that has shown that acute hypoxic gas breathing severely impaired task learning and cognition (Turner et al., 2015). Climbers at the summit of Mount Everest 29 029 ft. (8848m) have shown elevation in their alveolar-arterial oxygen difference, representing subclinical high-altitude pulmonary oedema or a functional limitation in pulmonary diffusion (Grocott et al., 2009). Short-term exposure to moderate hypoxia has been shown to decrease in performance in working memory related cognitive task (Zhang et al., 2011).

Magnetic resonance imaging (MRI) findings of resident at high altitude have exhibited structural modifications of their brains including loss of cortical grey matter with changes in white matter (Zhang et al., 2011) and greater left hemisphere activation more than right one (Yan et al., 2011). Electronic encephalography (EEG) findings of high altitude resident's brain have shown altered delta & beta frequency in resting state (Richardson et al., 2011). Both MRI and EEG findings provided an