EFFECTS OF TUALANG HONEY ON MEMORY PERFORMANCE, DEPRESSIVE-LIKE BEHAVIOUR, HISTOLOGICAL AND BIOCHEMICAL CHANGES IN YOUNG AND OLD MALE RATS EXPOSED TO LOUD NOISE STRESS

by

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LIST OF ABBREVIATIONS

µL	Micro litre
µM	Micro molar
µPa	Micro Pascal
5-HT	5-hydroxytryptamine
ACg	Anterior cingulate
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACTH	Adrenocorticotropic hormone
ADHD	Attention-deficit/hyperactivity disorder
AID	Dorsal agranular insular
AIV	Ventral agranular insular
ANOVA	Analysis of variance
AP	Ascorbate peroxidase
APP	Amyloid precursor protein
Aβ	β-amyloid
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
BDNF	Brain-derived neurotrophic factor
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
°C	Celcius
CA	Cornu ammonis
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CI	Confidence interval
CREB	Calcium/cyclic-AMP responsive element binding protein

CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
d2	Discrimination index
dB(A)	Decibels
DHEA-S	Dehydroepiandrosterone sulphate
DG	Dentate gyrus
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DTI	Diffusion-tensor imaging
EC	Entorhinal cortex
ELISA	Enzyme-linked immunosorbent assays
FA	Fractional anisotropy
FBW	Final body weight
FSH	Follicle-stimulating hormone
FST	Forced swim test
$GABA_B$	Gamma-aminobutyric acid B
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HPA	Hypothalamic-pituitary-adrenocortical
HPG	Hypothalamic–pituitary–gonadal
HRT	Hormonal replacement therapy
HDL	High-density lipoprotein
HNE	4-hydroxynonenal
IBW	Initial body weight

IIS	Insulin/IGF system
IL	Infralimbic
LH	Luteinizing hormone
LO	Lateral orbital
LTD	Long-term depression
LTP	Long-term potentiation
MAO	Monoamine oxidase
MD	Mean difference
MDA	Malondialdehyde
MO	Medial orbital
mPFC	Medial prefrontal cortex
MRI	Magnetic-resonance imaging
mtDNA	Mitochondrial DNA
NADPH	β-nicotinamide adenine dinucleotide phosphate
NE	Norepinephrine
NGF	Nerve growth factor
nm	Nanometre
NMDA	N-methyl-d-aspartate
NT	Neurotrophin
NORT	Novel object recognition test
O_2^-	Superoxide anion
OD	Optical density
OH	Hydroxyl radical
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
PCO	Protein carbonyl
PFC	Prefrontal cortex

PI3K	Phosphatidylinositol-3-kinase
PKC	Protein kinase C
PrC	Precentral
PrL	Prelimbic
PVN	Paraventricular nucleus
PWG	Percentage weight gain
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SOD	Superoxide dismutase
SEM	Standard error mean
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TMB	Tetrametyhlbenzidine
U	Unit
U/mL	Unit/mililiter
VLO	Ventral lateral orbital
VO	Ventral orbital
ZF	Zona fasciculata

KESAN MADU TUALANG KE ATAS PRESTASI INGATAN, TINGKAHLAKU SEPERTI KEMURUNGAN, PERUBAHAN HISTOLOGI DAN BIOKIMIA PADA TIKUS JANTAN MUDA DAN TUA YANG TERDEDAH KEPADA TEKANAN BUNYI BISING

ABSTRAK

Penuaan dan pendedahan kepada tekanan boleh menyumbang kepada kemerosotan ingatan dan kemurungan manakala stres oksidatif merupakan salah satu mekanisme yang mungkin terlibat. Pencarian penambahbaik fungsi kognitif daripada derivatif produk semulajadi yang mengandungi antioksidan semakin mendapat perhatian. Oleh itu, kajian ini bertujuan untuk memeriksa kesan penuaan dan tekanan ke atas prestasi ingatan dan tingkahlaku seperti kemurungan, dan menjelaskan kemungkinan mekanisme sandaran keberkesanan terapeutik madu Tualang dalam menambahbaikkan prestasi ingatan dan simptom kemurungan pada tikus jantan muda dan tua yang terdedah kepada tekanan bunyi bising. Sembilan puluh enam ekor tikus dibahagikan kepada lapan kumpulan: i) muda tanpa tekanan dengan plasebo, ii) muda tanpa tekanan dengan madu, iii) muda dengan tekanan dengan plasebo, iv) muda dengan tekanan dengan madu, v) tua tanpa tekanan dengan plasebo, vi) tua tanpa tekanan dengan madu, vii) tua dengan tekanan dengan placebo dan viii) tua dengan tekanan dengan madu. Suplementasi madu diberikan secara oral, 200 mg/kg berat badan selama 35 hari. Semua haiwan diuji dengan ujian pengenalan objek asing (NORT) dan ujian paksa renang (FST) sebelum suplementasi madu dan juga sebelum dan selepas prosedur tekanan bunyi. Apabila selesai menjalankan eksperimen, tikus dibius secara ringan dengan eter dan dibunuh serta-merta dengan pemenggalan dan sampel darah dan otak diambil. Hemisfera kanan otak dihomogenasikan dan

digunakan untuk mengukur aras/aktiviti enzim antioksida, penanda oksidatif, kapasiti antioksida keseluruhan (TAC), faktor neurotropik perolehan otak (BDNF) dan asetilkolinesterase (AChE) menggunakan kit asai komersial. Hemisfera kiri otak disimpan dalam formalin 10% untuk kajian histologi korteks prefrontal medial (mPFC) dan hipokampus. Aras kortikosteron, hormon adrenokortikotropik (ACTH) dan testosteron di dalam serum turut diukur dengan menggunakan kit asai komersial. Kajian ini mendapati bahawa terdapat kesan utama usia yang signifikan pada tingkahlaku seperti kemurungan, aras/aktiviti kortikosteron, ACTH, testosteron, TAC, SOD, GPx, GR, CAT, MDA, PCO, BDNF, AChE dan bilangan sel positif Nissl dalam mPFC dan kesemua bahagian hipokampus. Terdapat kesan utama tekanan yang signifikan pada prestasi ingatan, tingkahlaku seperti kemurungan, aras/aktiviti kortikosteron, ACTH, testosteron, TAC, SOD, GPx, GR, CAT, MDA, PCO, BDNF dan bilangan sel positif Nissl di dalam mPFC dan bahagian CA2 dan CA3 hipokampus. Suplementasi madu Tualang berupaya menambahbaikkan prestasi ingatan dan tingkahlaku seperti kemurungan, meningkatkan aras/aktiviti TAC, SOD, GR, BDNF, testosteron dan proliferasi neuron di dalam mPFC dan hipokampus, dan merendahkan aras/aktiviti MDA, PCO, kortikosteron, ACTH dan AChE. Kesimpulannya, madu Tualang berpotensi untuk digunakan sebagai terapi alternatif untuk mencegah daripada kemerosotan ingatan dan simptom kemurungan yang disebabkan oleh pendedahan kepada tekanan dan/atau penuaan.

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ABSTRACT

Ageing and stress exposure may lead to memory impairment and depression while oxidative stress is thought to be one of the underlying mechanisms involved. The search for cognitive enhancers from natural products derivatives possessing antioxidants has gained much attention. Thus, this study aimed to examine the effects of ageing and stress on memory performance and depressive-like behaviour, and to elucidate the possible mechanisms underlying therapeutic efficacy of Tualang honey in improving memory performance and depressive symptoms in young and old male rats exposed to loud noise stress. Ninety six male Sprague Dawley rats were divided into eight groups: i) unstressed young with placebo, ii) unstressed young with honey, iii) stressed young with placebo, iv) stressed young with honey, v) unstressed old with placebo, vi) unstressed old with honey, vii) stressed old with placebo and viii) stressed old with honey. The honey supplementation was given orally, 200 mg/kg body weight for 35 days. All animals were subjected to novel object recognition test (NORT) and forced swim test (FST) prior to honey supplementation as well as before and after the noise stress procedure. Upon completion of the experiment, rats were lightly anaesthetised with ether and immediately killed by decapitation, and their blood and brain samples were collected. The right brain hemisphere was homogenised and used to measure levels/activities of antioxidant enzymes, oxidative markers, total antioxidant capacity (TAC), brain-derived neurotrophic factor (BDNF)

and acetylcholinesterase (AChE) using commercially available assay kits. The left brain hemisphere was fixed in 10% formalin for histological study of medial prefrontal cortex (mPFC) and hippocampus. Serum corticosterone, adrenocorticotropic hormone (ACTH) and testosterone levels were also measured using commercially available assay kits. The present study demonstrated that there were significant main effects of age on depressive-like behaviour, levels/activities of corticosterone, ACTH, testosterone, TAC, SOD, GPx, GR, CAT, MDA, PCO, BDNF, AChE and number of Nissl-positive cells in the mPFC and all the hippocampal regions. There were significant main effects of stress on memory performance, depressive-like behaviour, levels/activities of corticosterone, ACTH, testosterone, TAC, SOD, GPx, GR, CAT, MDA, PCO, BDNF and number of Nissl-positive cells in the mPFC and hippocampal CA2 and CA3 regions. Tualang honey supplementation was able to improve memory performance and depressive-like behaviour, increase the levels/activities of TAC, SOD, GR, BDNF, testosterone and enhance the neuronal proliferation in the mPFC and hippocampus, and decrease the levels/activities of MDA, PCO, corticosterone, ACTH and AChE. In conclusion, Tualang honey has the potential to be used as an alternative therapy to protect against memory decline and depressive symptoms due to stress exposure and/or ageing.

CHAPTER ONE

INTRODUCTION

1.1 Cognitive functions of different parts of the brain

Cognitive function refers to a person's ability to process thoughts. Cognitive functions include problem-solving, speaking, learning, emotions, memory, perception and movement (Buckner and Wheeler, 2001). In most healthy individuals the brain is capable of learning new skills in each of these areas, especially in early childhood and of developing personal and individual thoughts about the world. Factors such as ageing and disease may affect cognitive function over time, resulting in issues like memory loss and trouble thinking of the right words while speaking or writing (Foster, 2006).

Limbic system is the combined neuronal circuitry that controls emotional behaviour and motivational drives. This large complex of brain structures is comprised of the hippocampal formation, amygdaloid complex of nuclei, hypothalamus, nucleus accumbens, cingulate cortex, ventral tegmental area, major areas of the prefrontal cortex and limbic midbrain areas (Morgane *et al.*, 2005). The limbic system is the home of emotions, motivation, memory regulation, the interface between emotional states and memories of physical stimuli, physiological autonomic regulators, hormones, "fight or flight" responses, sexual arousal, circadian rhythms and some decision systems. Figure 1.1 illustrates the gross anatomy of the human brain whereas Table 1.1 summarizes the functions of the major brain areas.

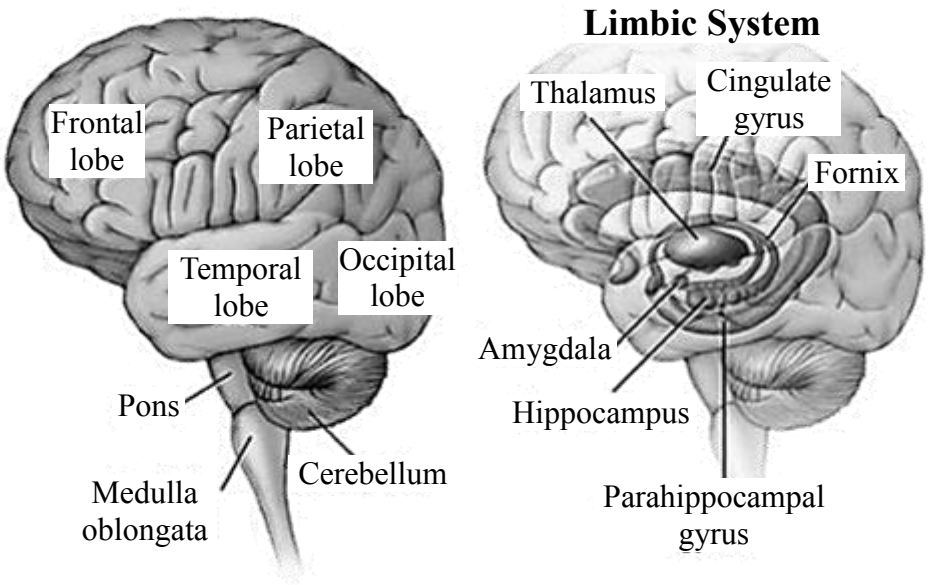

Figure 1.1 Gross anatomy of the human brain. Left panel shows the major lobes of the outer neocortex layer of the brain and right panel shows some of the major brain areas internal to the neocortex (Adapted from O'Reilly *et al.*, 2012)

Table 1.1 Summary of brain areas and their functions

Brain areas	**Functions**
Frontal lobe	Responsible for executive functioning and judgements, emotional response and stability, language, memory for habit and motor activities. Integral to personality, involved in tracking, sense of self, arousal and awareness environment
Parietal lobe	Involved in tactile (touch) perception, visual perception, integration of sensory information that allows for understanding of concepts and goal-directed voluntary movements
Temporal lobe	Plays key role in intellect, auditory perception (hearing), long-term memory and some visual perception
Occipital lobe	Visual perception system
Cerebellum	Involved in coordination and control of voluntary movement, balance and muscle tone
Brain stem	Plays role in heart rate, swallowing, reflexes to sight and sound, sweating, blood pressure, digestion, temperature, levels of alertness, ability to sleep and balance
Basal ganglia	Coordinate messages between multiple brain areas
The limbic system	
Thalamus	Relay centre for cortex; handles incoming and outgoing signals
Hypothalamus	Regulating body's homeostasis
Amygdala	Elicits emotion and aggression
Hippocampus	Involved in learning and memory

(Adapted from Barrett *et al.*, 2010)

1.1.1 The hippocampus as a cognitive map

The hippocampus is a part of the limbic system. In rat, the hippocampus occupies a large portion of the forebrain. Removal of the posterior and temporal neocortex of an animal such as the rat reveals the large sausage-shaped hippocampus underneath (Figure 1.2). For descriptive purposes it can be divided into a dorsal portion lying just behind the septum, a posterior portion where it begins to bend ventrally and laterally and a ventral portion lying in the temporal part of the brain (O'Keefe and Nadel, 1978). The hippocampus itself is divided into two major U-shaped interlocking sectors, the fascia dentata (*area dentata*, *dentate gyrus*) and the hippocampus proper (*cornu ammonis*). The part of the hippocampus visible on its dorsal aspect is the hippocampus proper, while the fascia dentata is buried inside and on the bottom surface of the sausage. Lorente de No (1934) divided the hippocampus proper into four fields, CA1-4, CA standing for cornu ammonis. The regio superior is called CA1 and the regio inferior is subdivided into CA2 and CA3. CA4 designated the scattered cells inside the hilus of the fascia dentate.

Cognitive maps (also known as mental maps, mind maps, cognitive models or mental models) are a type of mental processing composed of a series of psychological transformations by which an individual can acquire, code, store, recall and decode information about the relative locations and attributes of phenomena in their everyday or metaphorical spatial environment. Cognitive mapping is mainly believed to be a function of the hippocampus. The circuitry by which information arrives at and exits from the hippocampus is consistent with the idea that the hippocampus is important for both spatial and nonspatial memory. In both rats and macaques, detailed anatomical studies have indicated that spatial information arrives at the

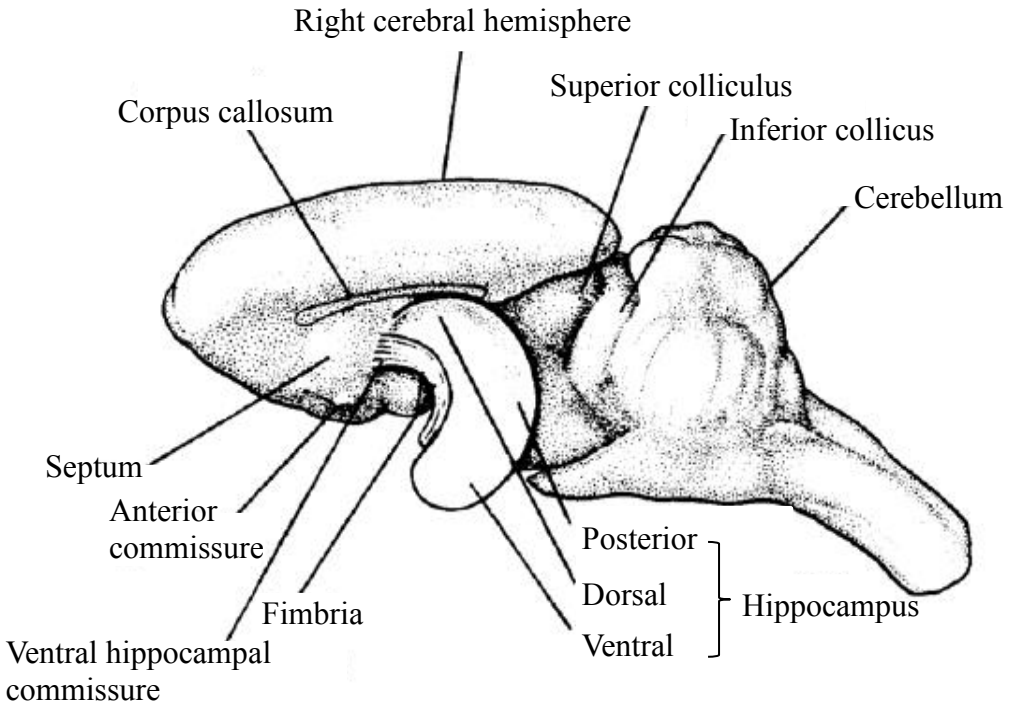

Figure 1.2 Drawing of the left rat hippocampus. All other forebrain structures except those at the mid-line have been removed (Adapted from O'Keefe and Nadel, 1978)

hippocampus via the postrhinal cortex (parahippocampal cortex in primates) and the medial entorhinal cortex, whereas nonspatial information takes a path largely through the perirhinal cortex and lateral entorhinal cortex (Witter and Amaral, 1991; Suzuki and Amaral, 1994; Witter *et al.* 2000). Thus, the hippocampus is ideally situated to combine spatial and nonspatial information in the service of remembering item–location associations (Manns and Eichenbaum, 2006). The integration of this information in the hippocampus makes the hippocampus a practical location for cognitive mapping.

The hippocampus plays an important role in spatial memory for both humans and rodents (O'Keefe, 1999; Burgess *et al.*, 2002). Lesions of the hippocampus lead to anterograde amnesia, or the inability to form or store new memories (Hall, 2010). Damage to the hippocampus has been implicated in the permanent loss of memory in patients with medial temporal lobe (which included the hippocampal formation, hippocampal gyrus, amygdala and uncus) resections (Scoville and Milner, 1957; Penfield and Milner, 1958; Milner, 1972). In line with clinical findings, combined amygdalohippocampal ablations in nonhuman primates have been shown to impair selective aspects of learning and memory capacities (Orbach *et al.*, 1960; Correll and Scoville, 1965; Mahut, 1971; Mishkin, 1978; Mahut *et al.*, 1979, 198l & 1982).

In more recent studies, the rodent hippocampus has been proven to be important for nonspatial memory (Eichenbaum *et al.*, 1999). Damage to the rat hippocampus leads to impairments on nonspatial tasks, including object recognition memory (Clark *et al.*, 2000; Fortin *et al.*, 2004), transitive odour associations (Bunsey and Eichenbaum,

1996), memory for temporal order (Fortin *et al.*, 2002; Kesner *et al.*, 2002) and social transmission of food preference (Alvarez *et al.*, 2001; Clark *et al.*, 2002).

1.1.2 The prefrontal cortex mediate executive function

The prefrontal cortex (PFC) is located in front of the premotor area of the frontal lobe and represents about a quarter of the entire cerebral cortex in the human brain. Based on Rose and Woolsey's (1948) definition of PFC as cortex in receipt of reciprocal connections from the mediodorsal thalamus, several distinct regions of PFC can be identified in the rat (Figure 1.3). The first is a medial frontal division, which can be subdivided into a dorsal region that includes precentral (PrC) and anterior cingulate (ACg) cortices and a ventral component that includes the prelimbic (PrL), infralimbic (IL) and medial orbital (MO) cortices. The second is a lateral region that includes the dorsal and ventral agranular insular (AID, AIV) and lateral orbital (LO) cortices. Finally, a ventral region can be delineated that encompasses the ventral orbital (VO) and ventral lateral orbital (VLO) cortices (Dalley *et al.*, 2004).

The PFC has been the focus of considerable scientific investigation in recent years, owing in part to the growing recognition that dysfunction of this region and associated circuitry probably underlies many of the cognitive and behavioural disturbances associated with major neuropsychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD) and schizophrenia. Patients with damage to the PFC show impaired judgement, organisation, planning and decision-making (Stuss and Benson, 1984), as well as behavioural disinhibition and impaired intellectual abilities (Elliott, 2003). In a laboratory setting, patients are impaired on tests such as set-shifting (Milner, 1963), planning (Shallice, 1982) and various

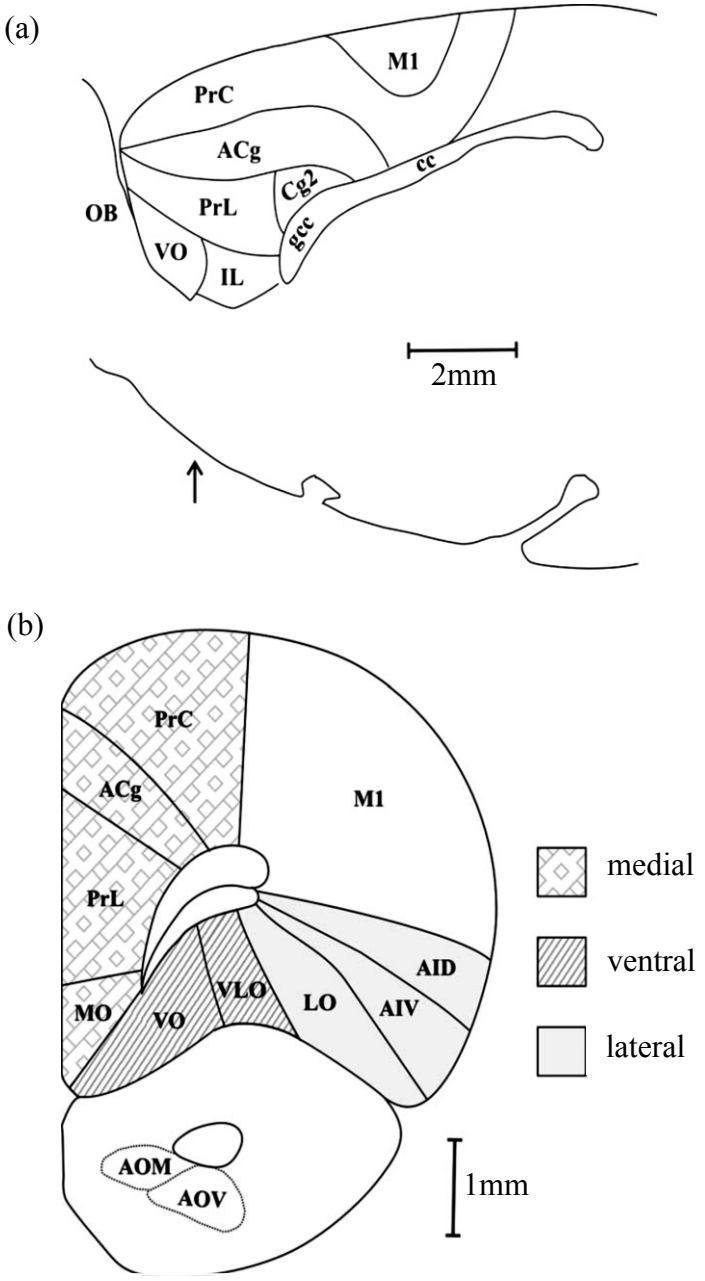

Figure 1.3 Illustrative diagrams of the rat prefrontal cortex (a) Lateral view, 0.9 mm from the midline. (b) Unilateral coronal section, approximately 3.5 mm forward of bregma. ACg, anterior cingulate cortex; AID, dorsal agranular insular cortex; AIV, ventral agranular insular cortex; AOM, medial anterior olfactory nucleus; AOV, ventral anterior olfactory nucleus; cc, corpus callosum; Cg2, cingulate cortex area 2; gcc, genu of corpus callosum; IL, infralimbic cortex; LO, lateral orbital cortex; M1, primary motor area; MO, medial orbital cortex; OB, olfactory bulb; PrL, prelimbic cortex; PrC, precentral cortex; VLO, ventrolateral orbital cortex; VO, ventral orbital cortex. (Adapted from Dalley *et al.*, 2004)

fluency tasks (Milner, 2002). These observations led to the influential conclusion that executive functions are the province of the PFC. The term executive function defines complex cognitive processing requiring the coordination of several subprocesses to accomplish a particular goal in a flexible manner (Funahashi, 2001). Studies in rats, monkeys and humans accord with the view that the PFC contributes to executive functioning (Baddeley, 1996; Fuster, 2000; Ongur and Price, 2000; Passetti *et al.*, 2000; Brown and Bowman, 2002). Table 1.2 summarized the rat PFC subregions and their mediation of specific processes associated with executive functions.

The PFC is also said to be the site of "working memory" (Hall, 2010). Working memory is the ability to hold and sort bits of information to be used in a problem-solving function. The function of the PFC, especially its dorsolateral part, includes short-term maintenance of retrieved or acquired information under the behavioural contexts that demand the execution of purposeful actions (Goldman-Rakic, 1995). A more general view holds that the PFC is critical for the 'on-line' maintenance of memory representations, which is necessary for the mediation of contingencies of action over time, especially under conditions of interference (Williams and Goldman-Rakic, 1995; Baddeley, 1996; Fuster, 2000; Miller, 2000; Brown and Bowman, 2002).

1.2 Physiological ageing

Physiological or non-pathological ageing is associated with a general decline of cognitive functions, such as declarative memory, verbal fluidity or working memory (Kramer *et al.*, 2004; Mahncke *et al.*, 2006; Stern, 2009). Most people experience a

Table 1.2 Rat prefrontal cortex subregions and mediation of processes associated with specific tasks

Tasks	ACg/PrC	PrL–IL/MO	AI/LO	VLO/VO
Working memory				
Responses	Yes	No	No	-
Objects and spatial locations	No	Yes	No	No
Odours and tastes	-	No	Yes	-
Temporal order memory				
Objects and locations	Yes	Yes	-	-
Memory for duration	No	-	-	-
Sequential processing	Yes	-	-	-
Prospective coding	Yes	Yes	-	-
Paired associate learning				
Visual-motor	Yes	-	-	-
Object-place	No	Yes	-	-
Odour-taste	-	-	Yes	-
Reversal learning				
Intra-modal	No	No	Yes	Yes
Cross-modal	No	Yes	No	-
Decision making				
Effort-based	Yes	-	-	-
Inter-temporal-based	-	Yes	Yes	Yes
Uncertainty-based	Yes	Yes	Yes	Yes

(Adapted from Kesner and Churchwell, 2011)

Note: Yes = subregional involvement, no = no subregional involvement, – = no available data. ACg/PrC, anterior cingulate/precentral cortices; PrL–IL/MO, prelimbic and infralimbic/medial orbital cortices; AI/LO, agranular insular/lateral orbital cortices; VLO/VO, ventrolateral orbital/ventral orbital cortices.

reduction in mental capacities with increasing age. This does not need to be related to disease processes, but occurs also as a result of normal ageing.

1.2.1 Factors that influence successful ageing

The World Health Organization (2003) has stressed that healthy ageing goes beyond avoidance of disease and disability. The term successful ageing has been defined by three main components: "low probability of disease and disease related disability, high cognitive and physical functional capacity and active engagement with life" (Rowe and Kahn, 1997). Other ways of defining successful ageing involve the degree to which elderly individuals adapt to age-associated changes (Schulz and Heckhausen, 1996; Baltes, 1997), view themselves as successfully ageing (von Faber *et al.*, 2001), or avoid morbidity until the latest time point before death (Fries, 2002).

As life expectancy continues to increase, it is important to understand the factors underlying the decline in memory and cognition that occurs with normal healthy ageing. Although cognitive function declines with age, there is considerable variability among individuals in the extent of this decline (Laursen, 1997). An important observation is that cognitive function of otherwise normal older individuals can be severely impaired shortly after experiencing a challenging life event such as an infection, surgery or psychological stress, an effect not readily observed in healthy young individuals (Wofford *et al.*, 1996; Bekker and Weeks, 2003; VonDras *et al.*, 2005). Thus, normal ageing is a vulnerability factor for the cognitive effects of these challenges (Tsolaki *et al.*, 1994; Laursen, 1997; Unverzagt *et al.*, 2001; Foster, 2006).

One of the common threads that have been found to correlate with successful ageing is the individual's socioeconomic status, particularly education and income levels. Higher educational attainment is associated with lower levels of negative affect, which is related to better health and increased life satisfaction (Meeks and Murrell, 2001). Material wealth and income have been shown to have a direct relationship to subjective well-being (Diener *et al.*, 1993). For many, the sense of well-being is especially affected by their feelings of income adequacy as they move into retirement. In addition, the access to surplus income allows for more recreation and less stress from financial concerns.

Various studies have associated religiousness with well-being, life satisfaction or happiness (Van Ness and Larson, 2002). Although it will be necessary for future research to more clearly specify which dimensions of religious participation are beneficial to which outcomes (Levin and Chatters, 1998), it appears that certain aspects of religious participation enables elderly people to cope with and overcome emotional and physical problems more effectively, leading to a heightened sense of well-being in late adulthood. Successful ageing is also associated with individuals' ability to develop and maintain strong social support systems (Rowe and Kahn, 1998). Solitude, or a lack of social interaction, is considered a major health risk factor (Unger *et al.*, 1999).

Maintenance of functional independence into old age likely has a genetic basis *vis a vis* findings that long life runs in families (Perls *et al.*, 2007; Westendorp *et al.*, 2009). Polymorphisms in specific gene loci, such as the components of the signaling pathways for insulin/IGF system (IIS) and for pituitary hormones, have been

identified as determinants of human longevity (van Heemst *et al.*, 2005; Suh *et al.*, 2008; Atzmon *et al.*, 2009; Pawlikowska *et al.*, 2009). Among the best examples are allelic variants of cholesterol ester transfer protein that are associated with maintenance of cognitive function among centenarians (Barzilai *et al.*, 2006; Sanders *et al.*, 2010). Other examples are the various polymorphisms within the IGF gene domain that are associated with overall maintenance of physical activity, high muscle performance and fat-free muscle mass among older adults (Schrager *et al.*, 2004; Hand *et al.*, 2007; Kostek *et al.*, 2010).

A summary measure of physiologic dysregulation, such as allostatic load, is an independent predictor of functional decline in elderly men and women (Karlamangla *et al.*, 2002). Allostatic load is described as a cumulative measure of physiologic dysregulation across multiple systems and was hypothesized to have considerable impact on future health risks (McEwan and Stellar, 1993). Allostatic load measures include systolic and diastolic blood pressure, waist-to-hip ratio, serum high-density lipoprotein (HDL) and total cholesterol levels, blood plasma levels of total glycosylated haemoglobin, serum dehydroepiandrosterone sulphate (DHEA-S) and urinary cortisol, norepinephrine and epinephrine excretion levels. In a 2.5-year longitudinal study, Seeman *et al.* (1997) found that allostatic load was associated with poor physical functioning at baseline, decline in physical functioning over time, poor cognitive performance at baseline and decline in cognitive performance over time. For instance, chronic elevations of cortisol levels lead to brain ageing (Landfield, 1987), hippocampal atrophy and cognitive impairment (Lupien and McEwen, 1997; McEwen *et al.*, 1999; Karlamangla *et al.*, 2005). These findings suggest that allostatic load is a risk factor for less successful ageing.

Physical activity may prevent or lessen age-related declines in physical functioning (Wagner *et al.*, 1992; LaCroix *et al.*, 1993; Shephard, 1993). In the Longitudinal Study of Ageing, physical activity was associated with the slope of decline in physical functioning over a six-year period; sedentary women experienced greater functional decline than those who were more active (Unger *et al.*, 1997). In addition, maintenance of an appropriate weight is associated with successful ageing. Obesity and underweight each has been associated with increased risk of physical impairment, as well as poor subjective health and well-being in the elderly (LaCroix *et al.*, 1993; Gillis and Hirdes, 1996).

1.2.2 Brain morphological changes in the course of ageing

The brain undergoes pronounced age-associated structural changes throughout life. According to multiple cross-sectional studies of healthy adults, advanced age is associated with smaller brains and thinner cortices (Raz *et al.*, 1997 & 2004; Jernigan *et al.*, 2001; Fjell et al, 2009a). Longitudinal investigations show that in healthy adults, brain parenchyma shrinks within a span of several years (Pfefferbaum *et al.*, 1998; Resnick *et al.*, 2003; Scahill *et al.*, 2003; Raz *et al.*, 2005; Driscoll *et al.*, 2009; Raz and Kennedy, 2009) and according to a recent report even in shorter periods of time (Fjell *et al.*, 2009b). Ageing is accompanied by various changes in the brain, such as cerebral atrophy (Raz, 2000), reductions in blood flow and metabolism (Newberg and Alavi, 1997) and neurochemical changes (Strong, 1998). Quantitative neuroimaging investigations have revealed moderate but consistent expansion of ventricular and cerebrospinal fluid (CSF) spaces across the adult life-span (Mu *et al.*, 1999; Resnick *et al.*, 2000; Jernigan *et al.*, 2001; Sullivan and Pfefferbaum, 2007).

The greatest age effect was commonly seen in the form of increased cortical sulcal, cerebral ventricular and cerebellar CSF (Jernigan *et al.*, 2001).

Both postmortem and *in vivo* neuroimaging studies have consistently shown that total brain volume decreases with increasing age (Jernigan *et al.*, 1991; Pfefferbaum *et al.*, 1994; Blatter *et al.*, 1995; Sullivan *et al.*, 1995; Murphy *et al.*, 1996; Raz *et al.*, 1997; Courchesne *et al.*, 2000; Resnick *et al.*, 2000; Good *et al.*, 2001; Jernigan *et al.*, 2001; Salat *et al.*, 2004; Sullivan *et al.*, 2004; Taki *et al.*, 2004; Allen *et al.*, 2005; Fotenos *et al.*, 2005; Walhovd *et al.*, 2005). The decrease in brain volume is caused by reductions in the volume of the cerebral cortex, white matter and several subcortical structures. In one study looking at 16 different brain structures, namely cortical gray matter, cerebral white matter, hippocampus, amygdala, thalamus, the accumbens area, caudate, putamen, pallidum, brainstem, cerebellar cortex, cerebellar white matter, the lateral ventricle, the inferior lateral ventricle and the 3rd and 4th ventricle, it was found that age is significantly correlated with all of the structures, except pallidum and the 4th ventricle (Walhovd *et al.*, 2005). Raz (2000) and Raz *et al.* (2004) suggested that the prefrontal cortices are more significantly affected than the rest of the neocortical regions. Practically all studies find thinning or volume reductions in frontal or prefrontal areas (Good *et al.*, 2001; Sato *et al.*, 2003; Salat *et al.*, 2004; Taki *et al.*, 2004; Brickman *et al.*, 2007; Raz *et al.*, 2007; Abe *et al.*, 2008; Kalpouzos *et al.*, 2009). In addition, the hippocampal volume shows moderately negative correlation with age, as do the amygdala, the cerebellum and the neostriatum (Raz *et al.*, 2004). Various other studies have reported significant age related decline in the volume of the hippocampus (Murphy *et al.*, 1996; Mu *et al.*, 1999) and amygdala (Mu *et al.*, 1999).

Various mechanisms might be responsible for the normative age-associated decline in brain structure, including hypertension, recurrent inflammation, age-associated vascular and microvascular changes, oxidative stress and stress-related corticosteroid levels (Whalley *et al.*, 2004). The effects of stress and oxidative stress on brain ageing are further discussed in succeeding subchapters.

1.2.3 Ageing and cognitive functions

As discussed above, it is clear that the brain undergoes large changes in structure and volume across the lifespan. Since cognitive processes are dependent upon the integrity of the brain, it seems probable that changes in brain morphology may decrease cognitive functions. There is ample evidence that alterations in brain structures are intimately tied to alterations in cognitive functions.

Larger brain volume may facilitate optimal cognitive abilities due to an extended number of neurons (Pakkenberg and Gundersen, 1997) and more synapses (Wickett *et al.*, 2000), thus allowing more differentiated and specialized afferent processing. Larger volume may also be accompanied with a higher neuronal complexity, an extended number of dendritic spines and more densely myelinated axonal walls (Deary and Caryl, 1997), which could allow for more complex, faster and more synchronous neuronal firing patterns. Therefore, reductions in total brain volume would likely cause declining of cognitive performance.

Several studies have shown that age-related volume loss of prefrontal structure principally results in deterioration of executive functions (Head *et al.*, 2005). The changes in frontal structure are responsible for cognitive problems often seen in older

people, such as attentional difficulties, forgetfulness and lack of cognitive flexibility. In addition, Kramer *et al.* (2007) found that a longitudinal decrease in hippocampal volume was associated with a decline in memory functions while a decline in cortical gray matter was associated with a decline in executive functions.

The parietal cortex and the precuneus (the posteromedial portion of the parietal lobe) are known to be involved in episodic memory retrieval (Buckner, 2004; Cavanna and Trimble, 2006). Finding by Salat *et al.* (2004) shows that an area dorsal in the left parietal cortex was thinner in elderly, while Lezak *et al.* (1995) proved that episodic memory reduced with increasing age. Thus, this suggests a possible structure-function correlation in ageing. In addition, precuneus anatomically projects to medial parietal cortex areas, i.e. posterior cingulate cortex and retrosplenial cortex, which also constitute a part of a cortical memory network (Buckner, 2004). Walhovd *et al.* (2006) have shown that cortical thickness in this area is related to verbal episodic memory over long time intervals, independently of hippocampal influence. Thus, volumetric changes in such parietal areas may contribute to changes in memory performance. Fjell *et al.* (2006) found that cognitively high-functioning elderly had thicker cortex in the posterior cingulate. These revelations also suggest a possible structure-function correlation in ageing.

The episodic memory network also involves frontal areas. Buckner (2004) suggests that the relatively mild memory problems often seen in physiological ageing is caused by gray matter atrophy and white matter changes in fronto-striatal systems, in addition to changes in corresponding neurotransmitter systems. As argued, frontal areas undergo significant structural changes with age. Walhovd *et al.* (2005) found

that the same was also true for striatum. Thus, this view also fits well with the observed volumetric changes in the brain in normal ageing. Walhovd *et al.* (2005) also reported a positive correlation between performance IQ and cortical volume and a negative correlation between P3a latency (as a measure of speed-of-processing) and cortical volume. Larger cortical volume predicted higher performance IQ and faster speed-of-processing.

Age-related changes in the white matter of the brain are assumed important for age-related changes in cognition. As discussed above, complex cognition involves the interplay between a large number of cortical areas and this requires the connections between the different brain areas to be efficient to allow fast signal transference between distant brain areas. Thus, if the brains nerve fibres are affected by normal age processes, this will likely exert effects on cognition. Recent studies show that white matter integrity as indexed by fractional anisotropy (FA; which is a marker for fibre integrity) has been reported to decline as a function of normal ageing (Moseley, 2002; Pfefferbaum *et al.*, 2005; Salat *et al.*, 2005; Charlton *et al.*, 2006; Sullivan *et al.*, 2006). White matter integrity may be especially important for cognitive processes that involve large neural networks, as is the case with working memory (D'Esposito, 2007). The decline of white matter integrity supports the disconnection hypothesis of normal ageing and accompanied cognitive decline (Moseley *et al.*, 2002; Charlton *et al.*, 2006; Grieve *et al.*, 2007).

Recent study also demonstrated a relationship between FA in frontal brain areas and executive functions (Grieve *et al.*, 2007). In addition, Salat *et al.* (2005) found that FA decreases were pronounced in prefrontal regions, which corresponds well both to

the known cortical volumetric reductions in the prefrontal cortex and to the reduced executive capabilities seen in elderly.

1.2.4 Hippocampal neurogenesis and cognitive ageing

In keeping with the historic view that the adult mammalian brain is incapable of adding new neurons, the addition of novel neural circuits has generally not been given consideration as a physiologically relevant mode of neural plasticity in adult mammals. This view, however, was challenged over 50 years ago when Joseph Altman demonstrated the presence of adult-generated granule neurons within the hippocampal formation of the adult rat (Altman, 1962 a,b). Since then, adult neurogenesis has been demonstrated in every major eutherian mammalian group in which it has been studied; rodents (Altman and Das, 1965; Angevine, 1965; Cameron and McKay, 1999 & 2001; Lavenex *et al.*, 2000; Snyder *et al.*, 2009), lagomorphs (Gueneau *et al.*, 1982), carnivores (Wyss and Sripanidkulchai, 1985; Hwang *et al.*, 2007; Cotman and Head, 2008; Amrein and Slomianka, 2010), tupaiids (Gould *et al.*, 1997; Simon *et al.*, 2005) and primates (Gould *et al.*, 1998; 1999 a & b; Kornack and Rakic, 1999; Leuner *et al.*, 2007; Perera *et al.*, 2007; Kordower *et al.*, 2010), including humans (Eriksson *et al.*, 1998; Knoth *et al.*, 2010), indicating that adult neurogenesis is a highly conserved, if not universal, feature among mammals.

New neurons in the dentate gyrus (DG) of the hippocampus project to CA3 and thus may form normal connections (Hastings and Gould, 1999) which presumably play a role in learning, memory and cognition. Evidence has been provided that newly produced neurons play an important role in functional and dysfunctional events

associated with the neurogenic area, such as memory function and neuropathologies (Abrous *et al.*, 2005). It has been proposed that a decline in hippocampal neurogenesis contributes to a physiologic decline in brain function. There is strong correlation between the number of new neurons and performance on hippocampal-dependent memory tasks such as the Morris water-maze and trace eye-blink conditioning (Shors *et al.*, 2001; Dobrossy *et al.*, 2003; Leuner *et al.*, 2004; Drapeau *et al.*, 2007; Drapeau and Abrous, 2008). Shors *et al.* (2001) observed a learning deficit in a hippocampal-dependent memory task after reducing the generation of new neurons with an anti-mitotic agent. Clear evidence for the functional role of neurogenesis has been provided by Imayoshi *et al.* (2008) who demonstrated that the genetic ablation of newly formed neurons in adult mice led to impairment in hippocampus-dependent cognitive function. Similarly, Jessberger *et al.* (2009) using a different genetic approach demonstrated that adult neurogenesis is correlated with spatial learning. In recent years, new and more complex methods have been designed to test the link between neurogenesis and cognition, revealing that neurogenesis is important for specific types of cognitive tasks.

It has been demonstrated that the rate of adult neurogenesis slows considerably with advancing age (Seki and Arai, 1995; Kuhn *et al.*, 1996; Simon *et al.*, 2005; Leuner *et al.*, 2007; Schoenfeld and Gould, 2012). The age-related decline in hippocampal neurogenesis, together with the decline in other type of synaptic plasticity, contributes to a physiologic decline in memory function (Bizon and Gallagher, 2003; Drapeau *et al.*, 2003). In a recent review, Drapeau and Abrous (2008) proposed that the differences in the risk of developing age-related memory disorders can be predicted earlier in life. Low hippocampal plasticity may render animals more

vulnerable to ageing processes. On the other hand, subjects starting off with a high level of neurogenesis may be resistant to the development of age-related memory disorders. In this regard, understanding the mechanisms that regulate the decline in neurogenesis in the aged hippocampus may guide the development of therapeutic strategies aimed at ameliorating age-related cognitive impairment.

In rodents, adult neurogenesis is present in the aged brain but is dramatically reduced in early adulthood in both the subventricular zone (Mirich *et al.*, 2002; Shook *et al.*, 2012) and the subgranular layer (Kuhn *et al.*, 1996; Cameron and McKay, 1999; Bernal and Peterson, 2004; Bondolfi *et al.*, 2004; Kronenberg *et al.*, 2006; Ben Abdallah *et al.*, 2010; Encinas *et al.*, 2011; Miranda *et al.*, 2012) of the DG. There is about 80% reduction in neuroblasts during the transition from young adult (2 months) to mid-age (7–9 months) in mice (Demars *et al.*, 2013) and a similar reduction from adult (4 months) to older (12 months) age in rats (Kuhn *et al.*, 1996; Nacher *et al.*, 2003; Rao *et al.*, 2006). After this period of dramatic reductions, the rate of decline is substantially reduced (Rao *et al.*, 2005) though the number of new neurons continues to decline (Demars *et al.*, 2013). This may manifest in deficits in olfactory and hippocampal-dependent function (Bizon *et al.*, 2004; Enwere *et al.*, 2004; Dupret *et al.*, 2008).

There are positive influences on neurogenesis that may be exploited in preventing the age-related decrease in neurogenesis. Training animals on a learning task enhanced hippocampus neurogenesis and the viability of the new neurons in the DG of rats (Gould *et al.*, 1999). Similarly, adult mice living in an enriched environment have increased neurogenesis in the hippocampus (Kempermann *et al.*, 1997). Moreover,

increased experience and social interaction led to an enhancement of neurogenesis in the DG of aged animals (Kempermann *et al.*, 1998). Finally, simple physical exercise (e.g. running) increased cell proliferation in the adult mouse DG (van Praag *et al.*, 1999). Hormonal factors also influence the rate of neurogenesis in ageing. For example, oestrogen has been demonstrated to stimulate a transient increase in neurogenesis in the DG of the adult female rat (Tanapat *et al.*, 1999). Furthermore, it was recently demonstrated that the level of neurogenesis typical of a young animal could be restored in an aged animal by decreasing the high levels of circulating corticosteroids that commonly occur in aged animals (Cameron and McKay, 1999).

1.2.5 Neurobiology of the ageing hippocampus

In young animals the hippocampus receives highly processed multi-modal information from widespread cortical association areas (Amaral and Witter, 1995). Sensory information enters the hippocampus largely via the perforant path, which projects from the entorhinal cortex (EC) to the DG, area CA3 and area CA1 (Figure 1.4). Outputs of the principle neurons in the DG project forward to CA3, then outputs of CA3 neurons project to the CA1 subregion and thence to the subiculum. Both CA1 and the subiculum are the origins of a major return pathway to the EC. Subtle reorganization of these hippocampal circuits during ageing might have a profound impact on memory processing.

It is said that the age-related volume loss of the hippocampus is a result of synapse loss (Terry, 2000; Hedden and Gabrieli, 2004). In aged rats, the DG receives approximately one-third fewer synaptic contacts from the EC than in young rats (Geinisman *et al.*, 1992; Smith *et al.*, 2000). The loss of input affects not only the

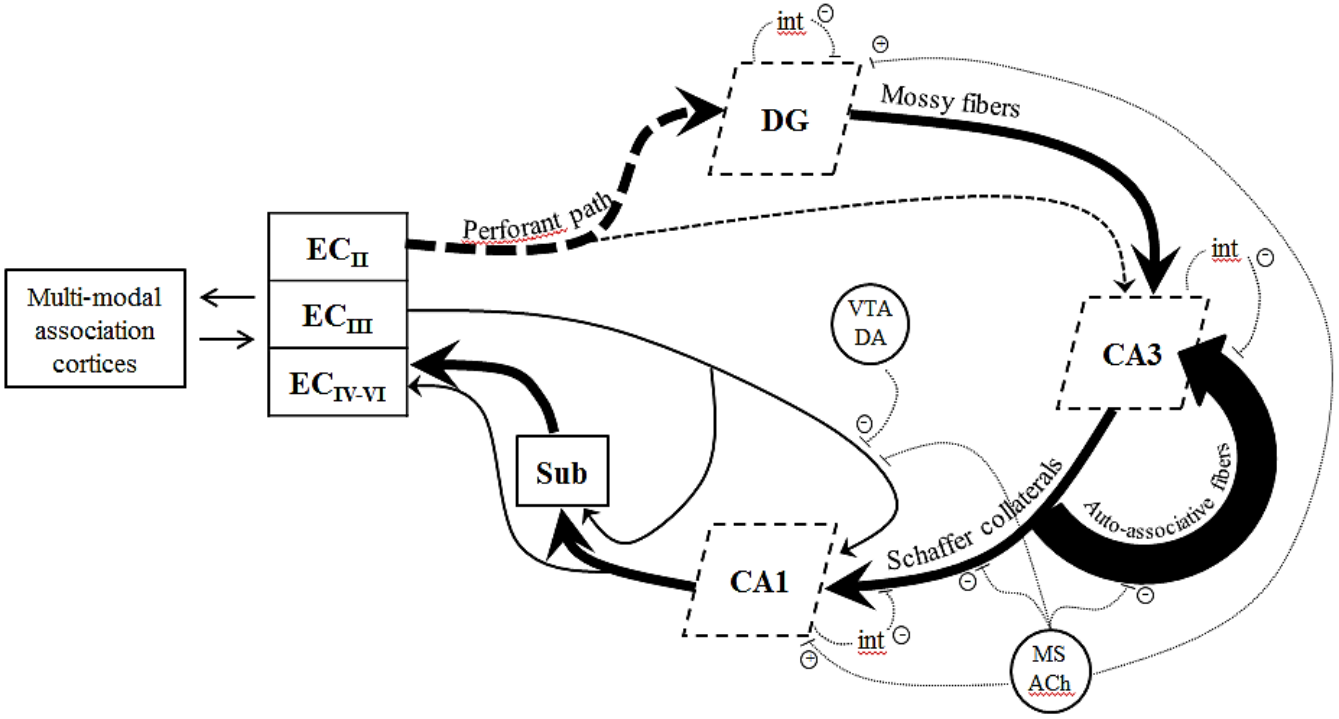

Figure 1.4 A neurobiological model of the aged hippocampus. Thick arrows represent trisynaptic pathway, including the CA1 outputs to the entorhinal cortex (EC) via the subiculum (Sub). Age-related deteriorations in functional connectivity are denoted by the broken lines. The following occurs with ageing: (i) the EC provides less input to the dentate gyrus (DG) and CA3, (ii) the medial septum (MS) provides less ACh-mediated modulation, (iii) interneuron (int) activity is decreased, (iv) the ventral tegmental area (VTA) provides less dopamine-mediated (DA) modulation and (v) the DG and CA1 are less excitable. These changes might increase the activity of CA3 recurrent collaterals (auto-associative fibres, thickest arrow) (Adapted from Wilson *et al.*, 2006)

connections of layer II EC neurons onto DG granule cells but also collaterals that feed forward onto CA3 pyramidal neurons (Smith *et al.*, 2000). Moreover, the extent of synaptic reduction correlates with the degree of spatial memory impairment in aged rats (Smith *et al.*, 2000). Corresponding to the loss of synaptic markers, a loss of cortical input has been detected electrophysiologically in studies showing that stimulation of the perforant path generates less excitation of the DG in aged rats than in young ones (Burke and Barnes, 2006).

Recent findings in elderly humans have demonstrated that aged subjects who have memory impairments possess a remarkably similar pattern of synapse loss in the perforant path. Scheff *et al.* (2006) reported that the number of synapses receiving EC input in the outer molecular layer of the DG was reduced in elderly subjects who had mild cognitive impairment compared with cognitively intact elderly subjects and the extent of synapse reduction correlated with memory ability. Furthermore, the use of diffusion-tensor imaging (DTI), a new magnetic-resonance imaging (MRI) technique, has revealed a marked atrophy in the perforant path of aged memory-impaired humans compared with young ones (Kalus *et al.*, 2006).

In young individuals, modulation by ACh is believed to be crucial for switching between modes of recall and storage within the hippocampus (Hasselmo *et al.*, 1995). The degree of modulation of the hippocampus mediated by ACh is attenuated in aged individuals and the extent of this loss correlates with the degree of memory impairment (Chouinard *et al.*, 1995; Nicolle *et al.*, 1999; Sugaya *et al.*, 1998). In addition, in young individuals, modulation by dopamine has a critical role in facilitating plasticity in CA1 hippocampus (Lisman and Grace, 2005). Dopamine