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An Investigation of the Effects of Exercise on Hippocampal Function in Young, Middle-aged and Aged Rats

by

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Thesis submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College

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2008

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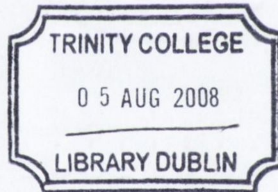
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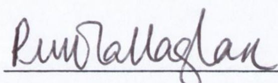

Rachel O'Callaghan

II Abstract

The hippocampus is a brain structure that is important for higher cognitive functions, possesses a high degree of plasticity, and is particularly vulnerable to the aging process (Burke & Barnes, 2006). Symptoms of hippocampal dysfunction and cognitive alteration are seen with both severe cognitive decline such as that associated with Alzheimer's disease and other forms of dementia (Small *et al.*, 1999). Given that demographic trends suggest the elderly population is increasing (Miller & O'Callaghan, 2005), it is especially important that interventions with the potential to improve cognitive function or ameliorate neurodegenerative decline are thoroughly investigated. Research has shown that in addition to improving general health and being associated with numerous benefits to the cardiovascular system, exercise positively affects neuronal function and has the capacity to improve cognitive function. Thus, exercise may be viewed as a simple means of maintaining brain function and promoting brain plasticity (Cotman & Berchtold, 2002). It is well-recognized that neurotrophins, most prominently brain-derived neurotrophic factor (BDNF), are central to the exercise-induced effects on brain function. Although research in this area is extensive, the precise mechanisms that underlie an exercise-induced improvement in cognitive function have yet to be identified.

The main aims of this research were to investigate the effects of exercise on hippocampal function in young, middle-aged and aged rodent populations, and to compare the effects of acute and chronic exercise protocols on cognitive function. In addition, the cellular modifications mediating an exercise-effect within the brain were explored.

Young (4 months), middle-aged (13-15 months) and aged (22-23 months) rats were used for these experiments. The acute exercise program consisted of 7 days consecutive running on a motorised treadmill for approximately 1km per day. The chronic exercise program consisted of 3 exercise sessions per week for 8 months. On completion of the exercise program, different forms of hippocampal function were assessed; long-term potentiation (LTP), object recognition memory or spatial learning. Biochemical analysis of brain tissue was completed to identify what cellular changes were mediating the effects of exercise on the brain.

Following acute exercise there was an enhancement in LTP and recognition memory in young animals. This exercise-induced effect was associated with an up-regulation of BDNF in the dentate gyrus. There was also an enhancement in LTP in aged animals with acute exercise. Within this population, the exercise-induced effect was associated with an increase in TrkB receptor expression and a concomitant decrease in p75 receptor expression. Acute exercise did not enhance spatial learning in young, middle-aged or aged animals. However, there was an improvement in spatial learning in middle-aged and aged animals following chronic exercise. In middle-aged animals, exercise-induced changes were associated with an increase in BDNF protein and altered NT4/5 mRNA expression in the dentate gyrus. In aged animals, exercise-induced changes were associated with a down-regulation in p75 mRNA expression in the dentate gyrus. Chronic exercise did not significantly improve LTP in either population. Interestingly, there was also evidence that aged animals specifically, may be responsive to environmental enrichment.

Collectively, these results show that exercise has the potential to enhance hippocampal function in both young and aged populations of rats. In addition, acute and chronic exercise may produce selective enhancements in hippocampal function. It is proposed that the cellular modifications underlying an exercise-induced effect may be specific to the type of hippocampal function, the type of exercise and age. Correlation analysis revealed a strong association between BDNF concentration in the dentate gyrus and LTP, thus highlighting its functional role with regard to hippocampal plasticity. Although the results support the theory that BDNF is an important player in mediating the effects of exercise on cognitive function, other factors are likely to be involved. It is speculated that other growth factors, namely IGF-1 and VEGF, may be increased with exercise, and that specifically within an aged population, exercise may have an anti-inflammatory effect.

III Acknowledgements

Although I have enjoyed the past few years battling with my PhD, at times it has been tough. I have been challenged to the maximus by this experience, and can honestly say that I would not have got to this stage were it not for the fantastic support of so many people. I feel very lucky to have been surrounded by such great family, friends, colleagues and mentors during the last 3 years.

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VI List of Abbreviations

A	Aged
ANOVA	Analysis of variance
Arc	Activity-regulated cytoskeleton-associated protein
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BDNF-LTP	Brain-derived neurotrophic factor-long-term potentiation
BrdU	5-bromo-2-deoxyuridine
BSA	Bovine serum albumin
CAMK	Calmodulin-dependent protein kinase
CBV	Cerebral blood volume
CNS	Central nervous system
CON	Control
COX	Cyclooxygenase
CREB	c-AMP response element-binding protein
DMSO	Dimethyl sulphoxide
DNA	De-oxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
EPSP	Excitatory post-synaptic potential
ERK	Extracellular signal regulated kinase
EX	Exercise
FRET	Fluorescence resonance energy transfer
IgG	Immunoglobulin G
IκB	Inhibitory κB
JNK	C-Jun N-terminal kinase
IGF-1	Insulin-like growth factor 1
Il-1β	Interleukin 1 beta
GABA	Gamma-aminobutyric acid
HFS	High frequency stimulation
HRP	Horseradish perioxidase conjugate
KA	Kainic acid

KCl	Potassium chloride
LTD	Long-term depression
LTP	Long-term potentiation
LPS	Lipopolysaccharide
MA	Middle-aged
MAPK	Mitogen-activated protein kinase
MCA	Middle cerebral artery occlusion
MEK	Mitogen-activated protein kinase kinase
MLK	Milk
MMP	Matrix metalloprotease
MRI	Magnetic resonance imaging
mRNA	messenger ribonucleic acid
MWM	Morris water maze
NE	North east
NF-κB	Nuclear factor-κB
NGF	Nerve growth factor
NMDA	N-methyl-D-asparate
NO	Nitric oxide
NT	Neurotrophin
NT3	Neurotrophin 3
NT4/5	Neurotrophin 4/5
NW	North West
OD	Optical density
p75	p75 low affinity neurotrophin receptor
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
pERK	phosphorylated ERK
PI-3K	Phosphatidylinositol-3 kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLC-γ	Phospholipase C-γ
pMCAO	Permanent middle cerebral artery occlsuion

proNGF	pro-neurotrophin NGF
proBDNF	pro-neurotrophin BDNF
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RQ	Relative quotient
RT	Room temperature
RT-PCR	Real-time polymerase chain reaction
SDS	Sodium dodecylsulphate
SE	South east
SEM	Standard error of the mean
SHR	Spontaneously hypertensive rats
SW	South west
TBS-T	Tris-buffered saline-tween
tERK	Total ERK
TMB	3,3' , 5,5' –Tetramethyl-benzidine
TNF-α	Tumour necrosis factor-α
Trk	Receptor tyrosine kinase
TrkA	Tyrosine kinase A
TrkB	Tyrosine kinase B
TrkC	Tyrosine kinase C
VEGF	Vascular endothelial growth factor
Y	Young

The most beautiful thing we can experience is the mysterious. It is the source of all true art and science.

-- Albert Einstein

Chapter 1

General Introduction

Chapter 1

1.1 Introduction

The hippocampus is a region of the brain believed to play a vital role in long-term memory and spatial navigation. It is important for higher cognitive functions, possesses a high degree of plasticity and is particularly susceptible to the aging process (Burke & Barnes, 2006). Consequently, the hippocampus is vulnerable to degenerative disorders of the aging brain. Symptoms of hippocampal dysfunction and cognitive alteration are seen with both severe cognitive decline such as that associated with Alzheimer's disease and other forms of dementia (Small *et al.*, 1999). Given that demographic trends suggest the elderly population is increasing (Miller & O'Callaghan, 2005) it is especially important that interventions with the potential to improve cognitive function or ameliorate neurodegenerative decline are thoroughly investigated. Promising research has shown that, in addition to improving general health, exercise positively affects neuronal function and has the capacity to improve cognitive function. In effect, exercise may be viewed as a simple means of maintaining brain function and promoting brain plasticity (Cotman & Berchtold, 2002). It is well reported that neurotrophins, most prominently brain-derived neurotrophic factor (BDNF), are central to the exercise-induced effects on brain function. Although research in this area is extensive, the precise mechanisms that underlie an exercise-induced improvement in cognitive function have not been elucidated.

1.2 Aging and Cognition

The aging process is accompanied by a general decline in many physiological systems e.g. endocrine, cognitive and motor. Quality of life can be significantly affected as the elderly face a variety of functional limitations that result from this deterioration. Specifically, dementias and other brain impairments may strongly impact normal daily living for both the aged individual and their close family. It is reasonable to assume that people are most fearful of the loss of self and an associated loss of cognitive capabilities that they know to be a possible affliction of age. Given that demographic trends suggest the elderly population is increasing (Miller & O'Callaghan, 2005), it is likely that the interest in this area of research

will continue to grow despite the extensive literature on brain aging that already exists.

1.2.1 The Hippocampus and the Aging Brain

The hippocampus is part of the forebrain, it is located in the medial temporal region of the brain and is considered crucial to behavioural regulation and activity-dependent learning and memory (Hwang *et al.*, 2006). The general arrangement of the hippocampus is similar for all mammalian species. The distinct subfields of the rodent hippocampus and projections within the hippocampal formation are shown in Figure 1.1. The hippocampus is part of the limbic system, which is a group of brain structures that has functions associated with emotion, behaviour and long-term memory. The limbic system also includes the cingulate cortex, the olfactory cortex and the amygdala.

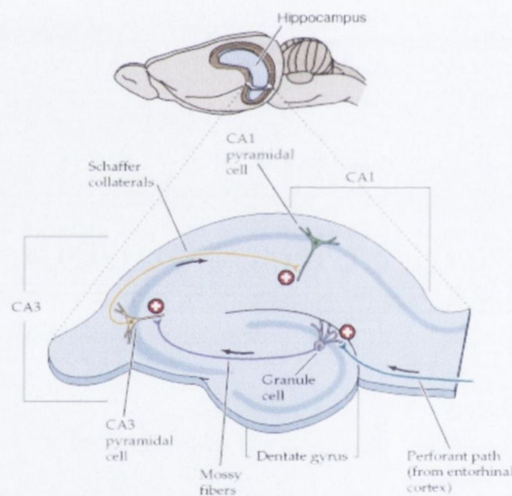


Figure 1.1 Rat hippocampus: image taken from Dale Purves (2004)
This illustration shows the location of the hippocampus within the rat brain. The subfields of the hippocampal formation and the projections from each region are also shown.

There are various types of learning that have been associated with the hippocampus e.g. spatial (Burger *et al.*, 2007; Ferbinteanu *et al.*, 2003), recognition (Hammond *et al.*, 2004), contextual (Gewirtz *et al.*, 2000) trace fear conditioning (Cuppini *et al.*, 2006) and episodic memory (Burgess *et al.*, 2002;

Smith & Mizumori, 2006).

Cognitive impairment is a natural consequence of age (Burke & Barnes, 2006). The hippocampus is particularly susceptible to the aging process (Burke & Barnes, 2006) and the aged hippocampus displays various functional and structural deficits. The age-related deterioration in learning and memory is associated with structural changes within the hippocampus. One such structural change is an age-related reduction of hippocampal volume (Driscoll *et al.*, 2006). Research has shown that spatial learning and memory, a form of hippocampal-dependent learning and memory that records information about our environment and spatial orientation, can be impaired with age (Fordyce & Wehner, 1993; Geinesman *et al.*, 2004; Greferath *et al.*, 2000). Barnes (2003) suggested that the age-related impairment in hippocampal-dependent forms of learning might be caused in part by altered mechanisms of synaptic plasticity (considered an activity-dependent process) within the hippocampus, including long-term potentiation (LTP).

Long-term potentiation (LTP) is a form of synaptic plasticity that has been proposed as a biological substrate for learning and memory. In 1966 Lomo observed that the response evoked in the dentate gyrus by a single test shock delivered to the perforant path, following a conditioning period of repeated test shocks, remained potentiated for a significant period of time. In 1973 Bliss and Lomo published a paper, considered definitive in the characterisation of LTP, that described long-lasting potentiation of synaptic transmission in the dentate gyrus following stimulation of the perforant path. The after-effects of repetitive stimulation of the perforant path fibres to the dentate gyrus were examined in anaesthetised rats. The results of the study suggested that two independent mechanisms are responsible for long-lasting potentiation: (a) an increase in the efficiency of synaptic transmission at perforant path synapses and (b) an increase in the excitability of the granule cell population. In support of the hypothesis that LTP and learning share common pathways, Richter-Levin and colleagues (1995) measured depolarization-induced release of endogenous glutamate in synaptosomes prepared from the dentate gyrus of anaesthetized rats and after training in the Morris water maze (MWM). The MWM is a task that was developed by Richard Morris in 1984 and is commonly used to assess spatial

learning in the rodent. Using environmental cues, the speed at which rats learn to escape from opaque water onto a hidden platform during repeated trials is recorded. Both spatial learning and LTP were accompanied by an increase in glutamate release from dentate synaptosomes. Additionally, Gooney and colleagues (2002) demonstrated parallel changes in the pre-synaptic terminal of the dentate gyrus with LTP and spatial learning. Specifically, phosphorylation of tyrosine kinase B (TrkB) and mitogen-activated protein kinase (MAPK), extracellular signal related kinase (ERK), was increased in rats that had undergone training in the MWM and had sustained LTP. These data cumulatively suggest that similar cellular modifications may underlie LTP and learning.

1.2.2 Mechanisms that underlie an age-related impairment in hippocampal function

While alterations in the expression of several signalling mediators may contribute to the age related-decline in LTP, the neurotrophin family of growth factors are a group of proteins that have been extensively investigated in this context. Gooney and colleagues (2004) suggested that the age-related reduction in LTP might be derived from impairment in the signalling of the neurotrophin BDNF in the hippocampus. In addition, Kelly and colleagues (2000) suggest that deficits in nerve growth factor (NGF) release and subsequent signalling in the dentate gyrus may contribute to age-related impairments in LTP.

Age-related decreases in other growth factors have also been associated with age-related impairments in cognitive function. Reductions in serum growth hormone and insulin-like growth factor 1 (IGF-1) have been proposed as mechanisms that may influence cognitive function in the elderly (Sonntag *et al.*, 2005). IGF-1 participates in vessel remodelling in the adult brain and it has been suggested that low serum/brain IGF-1 levels associated with old age and neurodegenerative disease may be related to an increased risk of vascular dysfunction (Lopez-Lopez *et al.*, 2004). In addition, Shetty and colleagues (2005) reported that the concentration of hippocampal vascular endothelial growth factor (VEGF) declines considerably by middle age. VEGF is an important signal protein involved in angiogenesis, the physiological process involving the growth of new blood vessels from pre-existing vessels. It is likely that a decrease in hippocampal VEGF may

equally contribute to vascular dysfunction in the aged brain.

Neurogenesis, the birth of new neurons, also declines precipitously with age (Kempermann *et al.*, 2002). Neurogenesis is intrinsically linked with different forms of brain plasticity. Although neurogenesis may be associated with the formation of some, but not all, types of hippocampal-dependent memories (Shors *et al.*, 2002), it has been speculated that adult hippocampal neurogenesis is a key factor in mediating functional hippocampal integrity in old age (Kempermann *et al.*, 2002).

The aged brain is also subject to inflammatory changes. Inflammation and its regulation by cytokines has been linked to many aspects of aging (Bodles & Barger, 2004). It has been reported that an interplay of pro-inflammatory and anti-inflammatory responses impact significantly on hippocampal synaptic function in the aged rat (Nolan *et al.*, 2005) and it is well recognised that inflammatory changes accompanying the aging process may negatively impact brain function, in severe cases causing neurodegeneration and cell loss. Age-related increases in interleukin-1β (IL-1β) concentration in the cortex and hippocampus have been coupled to age-related deficits in LTP (Martin *et al.*, 2002b). IL-1β was one of the first cytokines ever described. It is a pro-inflammatory cytokine primarily involved in immune defence against infection. IL-1β appears to play an important role in hippocampal synaptic function and may trigger apoptotic changes in the aged brain causing detrimental changes that are associated with neurodegenerative diseases (Lynch, 2002). Cacabelos and colleagues (1994) completed post mortem investigations and reported that demented patients had an increase in IL-1β production in the central nervous system; the highest concentrations of IL-1β were observed in brain regions, such as the hippocampus, where Alzheimer's disease neuropathology is most prominent.

1.2.3 Neurodegenerative Disorders

Severe cognitive decline is observed with various neurodegenerative disorders. Alzheimer's disease and other forms of dementia show symptoms of hippocampal dysfunction and cognitive alteration (Small *et al.*, 1999). Alzheimer's disease is of particular interest because it is the most common of the degenerative brain diseases (Selkoe, 2002). Alzheimer's disease is progressive and is characterised

by the accumulation of amyloid-β within vulnerable brain areas (Gouras, 2001). The deposition of amyloid-β-containing neuritic plaques and tau-containing neurofibrillary tangles are both considered defining lesions of Alzheimer's disease (Selkoe, 2002). It is believed that the underlying pathological process involves neuronal loss and inflammation. Consequently, there is a gradual deterioration of functions controlled by the affected parts of the nervous system. Clinical symptoms can include memory loss, disinhibition, disorientation and behavioural disturbances (Gauthier *et al.*, 1997; Gormley & Rizwan, 1998). The symptoms progress over time and at the latter stages of the disease mobility may be significantly affected leading to complete dependence on a carer. At least 10% of persons older than 65 years and 50% of those older than 85 years have some form of cognitive impairment, ranging from mild deficits to dementia (Yaffe *et al.*, 2001).

1.3 The Benefits of Exercise

The benefits of exercise to general health are well documented. One way in which aerobic exercise contributes to an improvement in general health is by reducing body weight, body mass index, body fat and intra-abdominal fat (McTiernan *et al.*, 2007). Exercise also reduces the risk of cardiovascular disease by controlling several atherosclerotic risk factors (Thompson, 2003). More specifically, exercise can improve arterial elasticity (Maeda *et al.*, 2005; Matsuda *et al.*, 1993) and decrease blood pressure (Miyatake *et al.*, 2003; Whelton *et al.*, 2002). Patients with ischemic heart disease can also greatly benefit from endurance exercise training. Ehsani (1987) observed that an improvement in left ventricular function was responsible for the increase in stroke volume and maximal oxygen uptake (a measure of aerobic capacity) following exercise training in patients with ischemic heart disease.

In addition to the cardiovascular benefits, research completed more recently has shown that exercise has a positive effect on neuronal function and has the capacity to improve cognitive function. Both intellectual and physical activity may be protective against neurodegenerative disease and the cognitive decline that can accompany aging (Miller & O'Callaghan, 2005). Given that exercise has the potential to ameliorate the mental decline associated with aging (Laurin *et al.*,

2001), exercise may be viewed as a simple means of maintaining brain function and promoting brain plasticity (Cotman & Berchtold, 2002).

1.3.1 Human Studies Investigating Exercise and Cognition

Human research investigating both normal and cognitively impaired populations has suggested that exercise may protect against dementia and Alzheimer's disease. Although previous research on the effects of exercise on cognitive impairment and dementia in old age was inconsistent, recent publications have more reliably reported a positive effect of exercise on cognitive function.

Stevens and Killeen (2006) completed a randomized controlled trial of nursing home patients with dementia and observed that a 12-week exercise program, specifically designed for an elderly population, slowed the rate of progression of the cognitive symptoms related to dementia. The experimental group exercised for 30min sessions, 3 times a week. Cognitive ability was assessed using the clock drawing test and the revised elderly disability scale. There are numerous versions of the clock drawing test and a number of scoring systems have been employed. All tests involve asking the individual to draw the face of a clock, include all the numbers and set the hands at a specific time e.g. ten past 11. This is a simple test that is commonly used as a screening tool for Alzheimer's disease and other forms of dementia. The latter assessment tool targets 8 areas of behaviour and psychosocial function to give a measure of cognition.

Heyn and colleagues (2004) performed a meta-analysis of existing data and found that exercise, in addition to improving fitness and physical function, increased cognitive function in people with dementia and related cognitive impairments. Rovio and colleagues (2005) reported that individuals participating in leisure-time physical activity, at least twice a week in midlife, were at a significantly reduced risk of developing dementia and Alzheimer's disease in later life than those who exercised less often. Abbott and colleagues (2004) studied elderly men who were cognitively normal and found that those who walked more were at a reduced risk of dementia. Cognitive function was assessed using the cognitive abilities screening instrument, a validated assessment tool, which focuses on 9 cognitive domains and is used clinically to determine the severity of dementia. Larson and colleagues (2006) demonstrated that regular exercise was associated with a delay

in the onset of dementia and Alzheimer's disease in an elderly population with no cognitive impairment.

Interestingly, although the majority of research focuses on the positive effects of aerobic exercise, it seems that resistance exercise or strength training, though less researched, may also have a positive effect on brain function. Cassilhas and colleagues (2007) demonstrated that moderate and high intensity resistance exercise programs lasting 24 weeks had equally beneficial effects on cognitive functioning in an elderly population.

There is convincing evidence that exercise positively affects cognitive function, either by reducing the risk of developing dementia and Alzheimer's disease, or by acting to ameliorate the cognitive impairment already present in those suffering from neurodegenerative decline. However, it is important to appreciate that the magnitude of fitness effects on cognition may be moderated by the length of the fitness-training intervention, the type of the intervention, the duration of training sessions and the gender of the study participants (Colcombe & Kramer, 2003).

1.3.2 Animal Studies Investigating Exercise and Cognition

Having identified a strong link between exercise and cognitive function by human research, the obvious advantage of animal work is that it enables investigation of the underlying molecular and cellular mechanisms involved. A wealth of animal research has been completed in an attempt to explain the neurological basis for an exercise-induced improvement in cognitive function. Numerous studies have shown that exercise programs can enhance LTP, improve spatial learning, increase neurogenesis and synaptic plasticity and also reduce stress-related atrophy.

Van Praag and colleagues (1999b) found that physical activity can increase neurogenesis, improve spatial learning and enhance LTP, thereby acting to regulate hippocampal function. Farmer and colleagues (2004) also demonstrated an enhancement of LTP in young adult Sprague-Dawley rats in response to a voluntary exercise program (minimum 1 week duration). Interestingly, weak theta-patterned stimulation that was unable to produce LTP in control animals produced robust and long-lasting LTP in exercised rodents. The authors proposed that there is an alteration in the induction threshold for synaptic plasticity in

animals that have engaged in voluntary exercise. It is speculated that this exercise-induced modification occurs in response to altered expression of BDNF and specific glutamate receptor subtypes in the dentate gyrus.

Anderson and colleagues (2000) showed that exercise influences spatial learning in 5-month-old Charles River rats. Exercising rats ran voluntarily for 7 weeks prior to and throughout testing in the radial arm maze. The results revealed that exercise can facilitate acquisition of a hippocampal-related spatial learning task, but does not affect performance following acquisition. Adlard and colleagues (2004) also observed an exercise-induced improvement in spatial learning in young adult Sprague-Dawley rats. Animals were given access to a running wheel for 3 weeks prior to, and during, assessment of spatial learning using the MWM. On day 2 of the task exercised animals had significantly shorter escape latencies than age-matched sedentary controls suggesting that learning acquisition was improved by exercise. Vaynman and colleagues (2004b) found that a short exercise period (1 week voluntary exercise) improved the performance of 3-month-old Sprague-Dawley rats in the MWM. Escape latency was significantly reduced on day 3 of training for the exercise group, where sedentary animals only showed a significant improvement in learning acquisition on day 4 of training. The results also showed that memory retention, assessed by a probe trial performed 2 days after the last MWM training day, was significantly greater in the exercise group compared with sedentary controls. This appears to be one of the only studies demonstrating an exercise-induced improvement in memory retention.

Radak and colleagues (2001) produced data showing that swimming training improves avoidance learning in rats. Avoidance learning is the process by which a behaviour or response in learnt in order to avoid an unpleasant experience. Young (4-week-old) and middle-aged (14-month-old) male Wistar rats were assigned to control and exercise groups. All exercising rats completed a swimming exercise program that involved swimming for 60min per day, 5 days a week for 9 weeks. The passive avoidance test showed that middle-aged exercised rats had significantly better short term (24hr) and long term (72hr) memory than age-matched control rats. Conditioned pole-jumping avoidance learning was markedly improved in both age groups in response to exercise. To assess active avoidance

the ability of rats to respond to unconditioned visual stimuli followed by conditioned stimuli 5secs later was measured.

Baruch and colleagues (2004) observed an enhancement in contextual fear conditioning following 30 days voluntary exercise in rats. Contextual fear conditioning is a form of learning that is believed to be hippocampal-dependent and also involves the amygdala (groups of neurons located deep within the medial temporal lobes of the brain that are believed to play a primary role in memory processing and emotional reactions). Given that this form of learning is considered hippocampal-dependent, this result provides further evidence that exercise alters hippocampal function and learning.

It is important to appreciate that while most research appears to focus on exercise-induced changes within the dentate gyrus, it is probable that exercise-induced cellular modifications in different subfields of the hippocampus, for example the CA1, may support changes in the dentate gyrus and other subfields, thus mediating the exercise-induced effects on plasticity and learning. It is also plausible that exercise-induced cellular changes specific to parts of the hippocampal formation may have different functional roles. In support of this, Okada and colleagues (2003) demonstrate that CA1 and dentate gyrus synapses play different functional roles in spatial learning, despite their similar mechanism for LTP induction. In addition, it is possible that an exercise-induced learning effect may affect other brain structures associated with the limbic system. Following 4 weeks treadmill exercise, Chen and colleagues (2008) observed a reduction in the concentration of serotonin in the hippocampus and the expression of its receptor in the amygdala of male rats. Serotonin is a neurotransmitter that is associated with a wide range of physiological systems and behavioural functions and may impair avoidance learning (Ogren *et al.*, 1985). Chen and colleagues (2008) proposed that, within the limbic system, down-regulation of the serotonin system is involved in exercise-enhanced fear memory.

An extensive exploration of the literature has clearly shown that exercise has beneficial effects on brain function. In some instances exercise-induced improvements in learning and memory are associated with enhanced neurogenesis but this link is not consistently reported. There is causal evidence that suggests some, but not all, hippocampal tasks require new neurons. Wojtowicz and

colleagues (2008), by inhibiting neurogenesis in adult rats, produced data that revealed neurogenesis is important for contextual fear conditioning but not spatial learning. Indeed, hippocampal-dependent learning may not always be associated with neurogenesis (Meshi *et al.*, 2006).

Having established a positive exercise-induced effect on cognitive functioning, it is important to recognize that while studies using human subjects predominantly assess the exercise-dependent effects on cognition by focusing on frontal-brain-dependent tasks (executive function), animal studies have commonly investigated the effects of exercise on hippocampal-dependent learning and plasticity.

1.4 Neurotrophins

Neurotrophins are a subclass of growth factors that were first discovered and described in the 1950s. This family of endogenous proteins regulate the development, maintenance and survival of new neurons (Lipton & Kalil, 1995). It has long been known that neurotrophins play a key role in the development of the nervous system (peripheral and central), but over the last decade critical effects of neurotrophins within the adult brain have also been identified. These growth factors provide trophic support to adult neurons and are closely involved in the expression of activity-dependent synaptic plasticity. Ultimately, they act to promote neurogenesis, neurite outgrowth and neurotransmitter release and increase synaptic sensitivity. However, individual neurotrophic factors may play different roles in neuronal plasticity (Castren *et al.*, 1993).

The neurotrophin family includes NGF, BDNF, neurotrophin 3 (NT3) and neurotrophin 4/5 (NT4/5). To date, the most extensively researched neurotrophins are NGF and BDNF. Neurotrophic factors signal by binding to two different types of transmembrane receptor proteins located on responsive neurons. The two classes of receptor are p75 and the Trk proteins, a family of tyrosine receptor kinases. The p75 receptor binds all neurotrophins with low affinity whereas the Trk receptors (TrkA, TrkB and TrkC) preferentially bind specific neurotrophins, but with much higher affinity. The neurotrophins bind with specific Trk receptors at the cell membrane (TrkA binds NGF, TrkB binds BDNF, NT3 and NT4/5, TrkC binds NT3). Binding activates the receptor which subsequently autophosphorylates within the intracellular domain. The phosphorylated tyrosines

act as protein interaction sites for molecules that initiate the activation of signalling pathways and ultimately the activation of transcription factors at the nucleus (Figure 1.2). The intracellular signalling initiated by neurotrophins can profoundly impact cell development and survival.

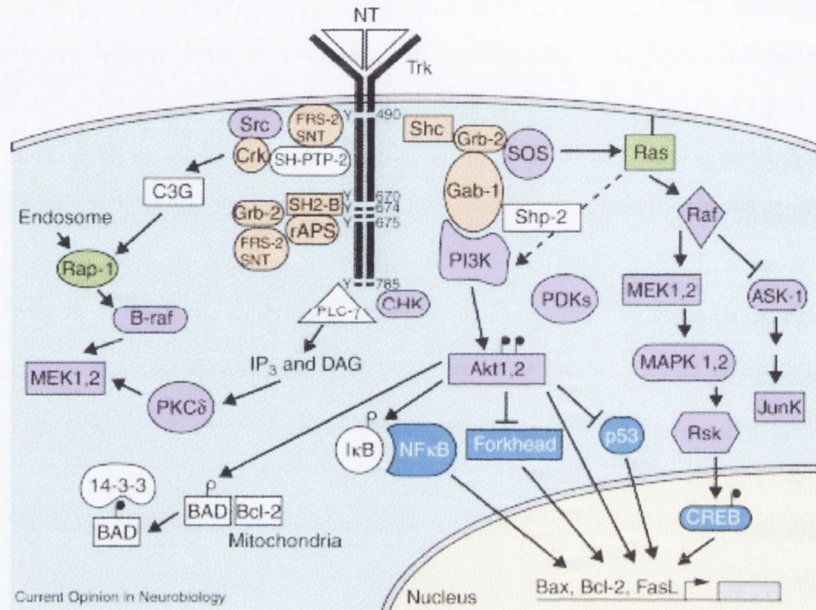


Figure 1.2 Trk signalling pathways: image taken from Patapoutin and Reichardt (2001)

The different intracellular signalling pathways associated with neurotrophins and Trk receptor activation are shown.

The neurotrophin hypothesis proposed by Schinder and Poo (2000) speculates that repetitive neuronal activity enhances the expression, secretion and/or actions of neurotrophins at the synapse to modify synaptic transmission and connectivity. Neurotrophins have been associated with LTP and hippocampal-dependent learning and are consistently reported to be up-regulated within the hippocampus following exercise. The theory that BDNF plays a central role in mediating the exercise-induced effects on brain plasticity is strongly supported (Molteni *et al.*, 2002; Vaynman *et al.*, 2003; Berchtold *et al.*, 2005).

1.4.1 Pro-neurotrophins

Pro-neurotrophins are precursors to the mature forms of neurotrophins. It was originally believed that proforms were cleaved intracellularly to release mature, secreted ligands and that the pro-neurotrophins were inactive precursors. However, it has more recently been suggested that the proforms of NGF and of BDNF may also be secreted and cleaved extracellularly to yield the mature forms (Lee *et al.*, 2001). This cleavage is performed by the serine protease plasmin and by selective matrix metalloproteinases. In addition, uncleaved pro-neurotrophins have been identified as active signalling molecules that activate the p75 neurotrophin receptor to mediate diverse responses (Hempstead, 2006). Lee and colleagues (2001) proposed that the biological action of neurotrophins is regulated by proteolytic cleavage with proforms preferentially activating the p75 neurotrophin receptor to mediate apoptosis. Thus, it seems that pro-neurotrophins and neurotrophins have opposing functional effects (Lu *et al.*, 2005).

1.4.2 BDNF; Synaptic Plasticity and Learning

BDNF is the most widely distributed growth factor found within the brain. It has a major influence on the survival and function of several neurotransmitter systems. BDNF promotes the survival of existing neurons and encourages the development (growth and differentiation) of new neurons. In the short term the actions of BDNF include an enhancement of neurotransmitter release and the ability to phosphorylate glutamate receptors at the synapse. Long-term actions involve the activation of signalling molecules and intracellular cascades and ultimately the alteration of gene expression profiles.

BDNF has been tightly linked with an increase in neurogenesis. Scharfman and colleagues (2005) proposed that BDNF induces neurogenesis by way of an enhancement in neuronal activity as well as having a direct action on neurogenesis. The actions of BDNF also contribute to an improvement in learning and memory.

In support of a link between BDNF and neuronal plasticity Korte and colleagues (1996) demonstrated that BDNF has an important functional role in the expression of LTP in the hippocampus. LTP was induced in hippocampal slices from mutant mice with a deletion in the coding sequence of the BDNF-gene. The mutants

showed significantly weaker LTP in the CA1. Adenoviral vectors, used to re-express BDNF in BDNF-knock-out-mice, successfully rescued LTP in most cases.

BDNF has also been shown to induce a protective effect against impairments in spatial learning and memory and LTP produced by chronic stress (Radecki *et al.*, 2005). Chen and colleagues (2007) demonstrated a link between BDNF and contextual learning. Contextual learning was shown to induce an increase in the number of hippocampal CA1 neurons expressing high levels of BDNF. A variety of stimuli that enhance neuronal activity may increase the expression of BDNF (Castren *et al.*, 1998; Gwag & Springer, 1993). In the context of Alzheimer's disease it has been suggested that a decrease in the expression of BDNF contributes to the progression of cell death in this disease (Phillips *et al.*, 1991). Given the apparent activity-dependent regulation of this molecule, a decrease in hippocampal BDNF would suggest a reduction in the afferent activity within the Alzheimer's hippocampus (Murray *et al.*, 1994).

1.4.3 Intracellular Signalling Targeted by BDNF

Trk receptor activation has been well-studied and it has been shown that the Trk receptors modulate synaptic strength and plasticity within the adult nervous system. Neurotrophins, by way of Trk binding and activation, mediate their effects through various intracellular signalling cascades (Figure 1.2). Activation of Trk receptors leads to the stimulation of a variety of signalling pathways including the well-characterized ras/ERK, phosphatidylinositol-3 kinase (PI-3K) and phospholipase C-γ (PLC-γ) pathways. Trk receptor-mediated signalling is controlled by the expression of intermediates in these signalling cascades and membrane trafficking that regulates the localisation of different signalling molecules (Huang & Reichardt, 2003). Although the p75 neurotrophin receptor has been shown to activate nuclear factor-kappa B (NF-κB), serine-threonine kinase Akt and J N-terminal kinase (JNK) pathways (Roux *et al.*, 2002) its significance *in vivo* has not been completely determined (Arevalo *et al.*, 2006). An important discovery in the field of p75 research was elucidation of the function of sortilin, a member of the Vps 10p-domain receptor family. It has been suggested that sortilin works as a co-receptor and molecular switch governing

p75-mediated apoptotic signalling induced by proNGF (Nykjaer *et al.*, 2004) and that the presence or absence of sortilin at the cell surface ultimately affects the way p75 expressing cells respond to pro-neurotrophin molecules (Arevalo & Wu, 2006). It has been suggested that the p75 receptor employs distinct signalling cascades that are in some cases synergistic with and in other cases are antagonistic with those activated by Trk receptors (Huang & Reichardt, 2003). Where Trk receptor activation is repeatedly shown to promote cell signalling that effectively leads to cell survival, differentiation, neurite outgrowth and activity dependent plasticity (Dan, 2004), p75 activation is more commonly associated with signalling that negatively impacts the cell (Kohn *et al.*, 1999).

Ultimately intracellular signalling converges at the nucleus where transcription factors are activated and gene expression is altered. Ribonucleic acid (RNA) is a nucleic acid polymer that serves as a template for translation of genes into proteins. Messenger RNA (mRNA) is RNA that encodes and transports information from deoxyribonucleic acid (DNA) during transcription to the sites of protein synthesis to undergo translation in order to yield a gene product. Through the activation and up-regulation of transcription factors gene profile expression and protein synthesis may be altered.

The actions of BDNF, through activation of the TrkB receptor, have been associated with the MAPK pathway, PI-3K pathway and activation of the protein kinase A/c-AMP response element-binding protein (PKA/CREB) signalling pathway. CREB is a transcription factor capable of binding DNA and regulating gene expression. In neurons, CREB proteins are involved in long-term memory formation and are necessary for late phase LTP.

Researchers have highlighted a link between BDNF/TrkB signalling and N-methyl-D-asparate (NMDA) receptors in hippocampal spatial memory. Mizuno and colleagues (2003) demonstrated the importance of an interaction between BDNF/TrkB signalling and NMDA receptors for spatial memory in the hippocampus. NMDA receptors are ionotropic receptors for glutamate. Activation of the receptor causes an ion channel to open and allows non-selective movement of small cations. Calcium flux through NMDA receptors located on the post-synaptic membrane may initiate changes in synaptic efficacy and promote pro-survival events (Vanhoutte & Bading, 2003) thereby suggesting that NMDA

receptors play an important role in modulating synaptic plasticity. Yamada and Nabeshima (2004) also reported that the interaction of BDNF/TrkB signalling and NMDA receptors is important for spatial memory and learning. In addition, they suggested that BDNF-dependent learning and memory was dependent on activation of the MAPK and PI-3K signalling pathways.

Endogenous BDNF has been implicated in the maintenance of high-frequency stimulation-induced LTP (Messaoudi *et al.*, 2002). Messaoudi and colleagues (1998) investigated the effect of an acute intrahippocampal infusion of BDNF on synaptic transmission in the dentate gyrus. Medial perforant path-evoked field potentials were recorded. BDNF infusion increased the field excitatory post-synaptic potential (EPSP) slope and population spike allowing the authors to conclude that exogenous BDNF induces a long lasting potentiation of synaptic efficacy in the dentate gyrus. Ying and colleagues (2002) found that BDNF-induced LTP in the adult hippocampus requires ERK activation coupled to CREB and up-regulation of activity-regulated cytoskeleton-associated protein (Arc), a calcium-regulated immediate early gene that is proposed to be involved in late LTP and long-term memory (Guzowski *et al.*, 2000). Ying and colleagues (2002) showed that BDNF-LTP was produced *in vivo* at medial perforant path-granule cell synapses; this cell population is one of the only major neuronal populations to undergo adult neurogenesis. BDNF infusion led to rapid phosphorylation of the MAP kinases ERK and p38. It was suggested that ERK activation is required for the induction but not the expression of BDNF-LTP, which was coupled to ERK-dependent phosphorylation of the transcription factor CREB. Results also revealed that BDNF infusion selectively upregulated the mRNA and protein for Arc. These data supports a model in which BDNF triggers long-lasting synaptic strengthening through MEK [MAPK kinase]-ERK and selective induction of the dendritic mRNA species Arc. Gooney and Lynch (2001) associated LTP in the dentate gyrus of the rat hippocampus with BDNF-induced activation of TrkB. The study reported that BDNF release is increased from slices of the dentate gyrus following high frequency tetanic stimulation (HFS) of the perforant path and TrkB activation is increased in synaptosomes prepared from tetanized dentate gyrus. These changes were accompanied by an increase in ERK activation. The authors also proposed that an increase in the phosphorylation of CREB and

protein synthesis may underlie the more long-lasting components of LTP.

Gottschalk and colleagues (1999) investigated the signalling mechanisms mediating BDNF modulation of synaptic plasticity in the hippocampus. Although this study assessed the developing hippocampus its still provides relevant insight into BDNF signalling mechanisms. Application of BDNF to neonatal hippocampal slices rapidly activated MAPK and PI-3K pathways. In addition, BDNF greatly attenuated synaptic fatigue at CA1 synapses. Repetitive stimulation (100Hz) of the Schaffer collaterals elicited a gradual decline of the slope and amplitude of EPSPs and was used to examine synaptic fatigue.

Alonso and colleagues (2002a) examined the role of BDNF in both short-term and long-term memory formation. The results showed that inhibitory avoidance training was associated with a rapid and transient increase in mRNA BDNF expression in the hippocampus. The authors concluded that endogenous BDNF is required for short-term and long-term memory formation of inhibitory avoidance learning. In addition, they proposed that ERK1/2-dependent mechanisms are involved. Specific to long-term memory formation they suggested that BDNF, in the CA1 region of the hippocampus, exerts its role, in part, via CREB activation and that BDNF-induced CREB activation seems to be primarily mediated through the activation of ERK1/2 signalling.

This exploration of the literature provides some indication of the extensive signalling associated with neurotrophins, specifically that of BDNF.

1.4.4 BDNF; Mechanism of Action

BDNF has important roles in modulating short-term synaptic function, activity-dependent synaptic remodelling and neurogenesis within the adult brain. Short-term actions are reported to be pre-synaptically mediated and include an enhancement in neurotransmitter release and the phosphorylation of glutamate receptors. Long-term actions involve post-synaptic mechanisms and include the activation of signalling molecules that subsequently activate various intracellular cascades and ultimately produce changes within the nucleus of the cell that impact learning and memory. It is probable that exercise-induced changes in gene expression explain how exercise induces long-lasting changes to modify synaptic structure and function, enhance neurogenesis and improve learning and memory.

In relation to neurogenesis it is possible that exercise-induced modification of gene expression profiles causes an up-regulation in proteins with important functional roles in cell proliferation and survival i.e. neurotrophins.

Xu and colleagues (2000) investigated the role of BDNF receptors in the expression of LTP in the mature hippocampus. Transgenic mice were used to explore the mechanisms by which BDNF produced an enhancement in LTP. Mutants in the BDNF receptor gene TrkB and antibodies to its other receptor p75 were used to determine which receptors and cells were involved in the LTP response. The results confirmed that BDNF plays a significant role in LTP and that it acts through the TrkB receptor. Inhibition of p75 did not reduce LTP or affect pre-synaptic function. The authors discussed whether the TrkB-mediated signalling relevant to LTP enhancement was pre-synaptic or post-synaptic. Cumulatively results suggested that a TrkB deficiency does not affect the properties of post-synaptic CA1 pyramidal neurons necessary for LTP production, which implies that BDNF acts pre-synaptically to modulate LTP in this brain region. It was hypothesized that BDNF signalling modulates the competence of pre-synaptic neurons to generate the repetitive exocytotic events needed to modify the long-term responses of post-synaptic neurons.

Tyler and colleagues (2002) reviewed the role of BDNF signalling in hippocampal-dependent learning and concluded that BDNF/TrkB signalling plays a fundamental role in mediating enduring changes in central synaptic structure and function. The authors also discussed the existing theories in relation to how BDNF mediates its effects. It has been suggested that BDNF modulates pre-synaptic plasticity by way of BDNF/TrkB signalling but also exerts significant effects on post-synaptic function. Pre-synaptically, BDNF induces a profound enhancement of neurotransmitter release. Changes in post-synaptic responsiveness to glutamate release may include enhanced glutamate receptor function and modulation of voltage-gated ion channels. It is speculated that both pre-synaptic and post-synaptic mechanisms may underlie the role of BDNF in learning and memory.

Specifically in the context of exercise, Molteni and colleagues (2002) suggested that an exercise-induced elevation in the expression of BDNF can affect neuronal plasticity by acting at both pre- and post-synaptic terminals. It was reported that

TrkB signalling at the pre- or post-synaptic membrane can result in up-regulation of several downstream genes, members of the MAPK pathway and calcium/calmodulin kinase (CAMK) family. With respect to pre-synaptic changes it was proposed that exercise can modulate the expression of genes associated with synaptic trafficking to promote neurotransmitter release.

A study completed by Adlard and colleagues (2004) investigated the time-course of induction of BDNF mRNA and protein after 1, 3, 5, 7, 14 and 28 days of exercise in the rat. BDNF protein was significantly increased after 4 weeks of exercise, where the mRNA for individual BDNF exons significantly increased over the time-course of the study. This result is particularly interesting in regard the theory that there may be different waves of activation of BDNF.

1.5 Exercise-induced Changes in BDNF

Given that BDNF has been intrinsically linked with cognitive function it follows that if exercise up-regulates BDNF it may, by enhancing trophic support in key brain areas, provide a simple tool for ameliorating the decline in brain function caused by neurodegeneration.

Russo-Neustadt and colleagues (1999) observed that voluntary physical activity for a 20-day period produced an enhancement in BDNF up-regulation resulting from antidepressant treatment in Sprague-Dawley rats. Exercise alone also induced an increase in mRNA BDNF in various brain regions (CA1, CA3, CA4 and dentate gyrus). Neeper and colleagues (1996) observed a significant increase in BDNF mRNA in several brain areas, most notably within the hippocampus and caudal 1/3 of the cerebral cortex, following 2, 4 and 7 nights of exercise. Tong and colleagues (2001) investigated the impact of exercise on gene-expression profile in the rat hippocampus. Young Sprague-Dawley rats exercised for 3 weeks prior to sacrifice. Exercise was shown to influence a large number of gene transcripts, many of which are known to be associated with neuronal activity, synaptic structure and neuronal plasticity. Of particular relevance was the result that levels of mRNA BDNF were increased in the hippocampus following exercise. Widenfalk and colleagues (1999) observed an increase in the expression of mRNA coding for BDNF and its high affinity receptor TrkB in the hippocampus in a running-length-dependent manner. Spontaneously hypertensive

rats, known to run for up to 20km/night, were housed with free access to running wheels. Exercising rats engaged in physical activity for either 6 weeks controlled exercise (exercise level was controlled by locking the running wheels once the set distance of 3km/8km had been reached) or 5 weeks free running. The expression of BDNF and TrkB mRNA increased in the hippocampus with exercise; this increase was proportional to the amount of exercise. A simultaneous up-regulation of both ligand and receptor could presumably potentiate paracrine or autocrine modes of stimulation. Interestingly, an abrupt interruption of prolonged spontaneous exercise decreased the expression of BDNF and TrkB mRNA in specific hippocampal regions (the most prominent reduction was evident in the medial cornu ammonis, CA3M) for at least 10 days. It is possible that exercise-induced alterations in synaptic plasticity that are mediated by BDNF are specific to regions of hippocampal circuitry. No robust changes in response to exercise or an abrupt interruption of prolonged spontaneous exercise were observed with other members of the neurotrophin family, NGF and NT3. Interestingly, Adlard and colleagues (2004) demonstrated that exercise has the ability to modulate BDNF induction in a time-dependent manner. Adult male Sprague-Dawley rats (2 months) were given access to a running wheel for 1, 3, 5, 7, 14 and 28 days. A significant increase in hippocampal BDNF protein was seen after 4 weeks of voluntary exercise where the mRNA for individual BDNF exons increased significantly over the time-course of the study.

Having established that exercise up-regulates BDNF Berchtold and colleagues (2005) investigated (a) whether exercise-induced effects were similar with intermittent exercise and (b) whether the changes in BDNF expression induced by exercise persisted following cessation of exercise. BDNF protein expression was used as an index of the hippocampal response to exercise. To investigate the effect of different training paradigms on BDNF levels young adult Sprague-Dawley rats were exercised under two conditions of voluntary wheel-running, continuous daily access for 2, 4, 7, 14, 28, or 90 days or intermittent access on alternating days for 7, 14, 21, or 28 days. Both daily exercise and alternating days of exercise increased BDNF protein. Levels were also seen to progressively increase with longer running duration. Daily exercise and exercise on alternate days were equally effective at increasing BDNF. In addition, the results revealed

that BDNF protein remained elevated for several days after exercise ceased. Interestingly, the authors also reported that, after prior exercise experience, a brief second exercise re-exposure insufficient to cause a BDNF change in naïve animals, rapidly reinduced BDNF protein to levels normally requiring several weeks of exercise for induction. This occurred with an intervening "rest" period of up to 2 weeks and suggests that exercise primes a molecular memory for BDNF induction. The results from this study are of particular relevance clinically because they provide valuable insight for optimising the design of exercise and rehabilitation programs that may be used to enhance hippocampal function.

There is strong evidence that exercise may up-regulate BDNF. Few studies have associated exercise-induced increases in BDNF with exercise-induced improvements in hippocampal function. Shaw and colleagues (2003) observed that 4 days of prior exercise in a running wheel reversed experimentally-induced inhibition of LTP and spatial learning. Cyclooxygenase, (COX) plays a crucial role in inflammation. The broad-spectrum COX inhibitor ibuprofen was used to inhibit the induction of LTP and caused substantial and sustained deficits in spatial learning in a group of young adult Wistar rats. Exercise for a minimum of 100m per night on 4 consecutive nights prior to experimental manipulation increased endogenous BDNF levels in the dentate gyrus sufficiently to reverse the effects of broad-spectrum COX inhibition. In addition, Vaynman and colleagues (2004b) found that a 1-week voluntary exercise period enhanced spatial learning and was associated with an increase in the mRNA levels of BDNF and its TrkB receptor. A specific immunoadhesin chimera (TrkB-IgG) that mimics the BDNF receptor TrkB to selectively bind BDNF molecules was used to block BDNF in the hippocampus. Inhibiting BDNF blocked the effect of exercise on cognitive function. This result was cited as the first direct evidence to show that exercise may predominantly employ the action of BDNF to enhance cognitive function.

It is well-reported that the exercise up-regulates BDNF and in some instances exercise-induced effects on brain function have been linked to an increase in BDNF. Specifically in the context of neurodegeneration, an exercise-induced up-regulation of BDNF may help increase the brain's resistance to age-related damage and neurodegeneration (Oliff *et al.*, 1998).

1.5.1 Exercise-induced BDNF Signalling

Where exercise-induced effects on cognitive function have been associated with BDNF, a number of associated signalling cascades have been identified. Chen and Russo-Neustadt (2005) demonstrated that exercise activates the PI-3K pathway. Young adult male Sprague-Dawley rats completed 14 days of daily voluntary wheel running. The results of the study suggested that the exercise-induced increase in BDNF was associated with an increased expression of several key intermediates of the PI-3/Akt pathway, known for its role in enhancing neuronal survival. Hippocampal expression of PI-3K and phosphorylation of protein-dependent kinase-1, thr308-Akt, CREB and Trk were increased in exercising animals. A subsequent study by this group (2006) confirmed their previous results and additionally suggested that nitric oxide synthesis is required to produce exercise-induced increases in hippocampal BDNF and PI-3K expression. Nitric oxide (NO) is an important signalling molecule that has neuronal survival-promoting properties (Cheng *et al.*, 2003) and has been shown to play an important role in plasticity. The results suggest that the marked enhancement in BDNF mRNA and PI-3K expression that occurs with exercise is strongly dependent on NO signalling.

Specific to an exercise-induced effect, Vaynman and colleagues (2004b) found an association between the expression of BDNF and CREB and cognitive function. These results suggested a functional role for BDNF-regulated CREB in mediating improvements in learning and memory induced by 1 week of voluntary exercise. The suggestion that the transcription factor CREB may be increased as a result of exercise implies that gene expression may be altered. It follows that a change in specific gene transcripts would ultimately affect neuronal activity, synaptic structure and neuronal plasticity all of which would influence brain function, perhaps more notably in the long term. Vaynman and colleagues (2004a) also revealed that BDNF has the ability to mediate exercise-induced hippocampal synaptic plasticity by regulating the synaptic vesicle protein synapsin I in specific hippocampal subfields. Quantitative immunohistochemical analysis showed increases of BDNF and synapsin I in the CA3 stratum lucidum and dentate gyrus and increases in synapsin I alone in the CA1 stratum radiatum and stratum laconosum moleculare following exercise. The authors speculate that exercise

induces plasticity of select hippocampal transsynaptic circuitry, possibly comprising a spatial restriction on synapsin I regulation by BDNF.

Following from this, Vaynman and colleagues (2005) suggested exercise regulates specific properties of synaptic transmission under the direction of BDNF. A group of molecules involved in synaptic transmission were investigated (synapsin I, synaptophysin and syntaxin). These molecules are known to have distinct actions on vesicle clustering, endocytosis and exocytosis. The authors speculated that these actions were key events characterising synaptic function during exercise. Adult male Sprague-Dawley rats were given access to a running wheel and completed 3 days of voluntary exercise. The results demonstrated that exercise up-regulated BDNF selectively modulating the levels of synapsin I and synaptophysin, but not syntaxin, within the hippocampus, thus highlighting the capacity of exercise to modulate specific properties of BDNF-mediated synaptic plasticity.

Shen and colleagues (2001) attempted to explain the molecular mechanisms involved in the physical activity-induced beneficial effects on brain function. The study investigated the influence of voluntary exercise on the activation of signalling molecules (CREB and MAPK/ERK) that are known to play vital roles in synaptic plasticity, including learning and memory. Voluntary exercise in young adult male Sprague-Dawley rats increased the level of activated CREB and phosphorylated MAPK. The exercising period was either 1 night, 3 days, 7 days, 1 month, or 2 months. The time-course of increase was different for the two molecules. Interestingly, in comparison with the activated CREB, where levels doubled after 1 night of running and control levels were restored after 1 month of exercise, the increase in phosphorylated MAPK was delayed but lasted longer and increased levels were still detectable after 1 month of exercise. The relatively sustained increase in the activation of these signalling molecules in response to exercise supports the view that this signalling induces lasting, beneficial changes in brain function through the expression of target genes i.e. BDNF.

1.6 Exercise-induced Changes in NGF

Most research has established BDNF as a central mediator of the exercise-induced effects on synaptic plasticity and cognition. However, some literature has shown

that NGF also contributes to and is involved in promoting the exercise-induced effects on the brain.

Ang and colleagues (2003) investigated the possibility that neurotrophins, namely NGF, may be mediating a neuroprotective effect of exercise. A 12-week exercise program that involved treadmill running reduced the volume of infarction caused by middle cerebral arterial occlusion in young male Wistar rats. The results of the study suggest that neuroprotection induced by physical exercise may be a result of endogenous NGF and the proliferation of its receptor cholinergic neurons.

A study by Neeper and colleagues (1996) that has been previously discussed demonstrated a significant increase in hippocampal mRNA NGF after 2 nights voluntary wheel running. Significant increases in mRNA NGF were also observed in the caudal neocortex after 2, 4 and 7 nights of exercise.

1.7 Exercise and IGF-I

IGF-I is a polypeptide hormone that promotes cell growth and multiplication and has been implicated in neurogenesis. Lindholm and colleagues (1996) demonstrated the complementary action of IGF-I and BDNF in supporting survival by providing evidence that both IGF-I and BDNF increased the survival of hippocampal neurons lacking BDNF during development. Aging is accompanied by reductions in both hippocampal neurogenesis and IGF-I levels. Anderson and colleagues (2002) reported that systemic infusion of IGF-I increases both proliferation and neurogenesis in the adult rat hippocampus. Furthermore, IGF-I replacement in aged animals increases neurogenesis, glucose utilization, vascular density and alters NMDA receptor subunit composition in brain areas implicated in learning and memory (Sonntag *et al.*, 2005). Trejo and colleagues (2004) also reported that serum IGF-1 contributes to brain amyloid-β clearance and affects learning and memory. Thus, changes in levels of IGF-I may be associated with several neurodegenerative illnesses. Trejo and colleagues (2004) hypothesized that in the majority of neurodegenerative diseases compromised IGF-I support to neurons emerges as part of the pathological cascade during the degenerative process and contributes to neuronal demise.

Given there is a well-established link between IGF-1 and neurogenesis it seems a reasonable assumption that IGF-1 may be contributing to the exercise-induced

improvement in cognitive function. Furthermore an up-regulation of IGF-1 may help ameliorate some of the neurodegenerative decline associated with aging. Carro and colleagues (2001) demonstrated that the positive effects of exercise on brain function are mediated by way of an increase in uptake of circulating IGF-I by the brain. Laboratory rodents were subjected to various neurotoxic insults either before or after treadmill running. Three models of experimental neurodegeneration affecting different brain regions were used to determine whether exercise offers neuroprotection to all types of neuronal populations. Partial lesions were induced in the hippocampus, brainstem and cerebellum. The results indicate that exercise prevents damage through increased uptake of circulating IGF-I by the brain. The authors proposed that exercise is neuroprotective and not only attenuates the impact of a brain insult but also impedes progression of ongoing neurodegeneration.

Further work completed by Carro and colleagues (2000) confirmed that circulating IGF-I mediates the effects of exercise on the brain. In this study it was reported that forced running induced the uptake of blood IGF-I by specific groups of neurons, cerebellar purkinje cells and dorsal column nucleus cells, throughout the brain. Prior to exercise animals were injected with dioxigenin-labeled IGF-I either in the carotid artery or in the lateral cerebral ventricle. Brain uptake of IGF-I after either intracarotid injection or after exercise elicited the same pattern of neuronal accumulation of IGF-I suggesting that systemic injection of IGF-I mimicked the effects of exercise on the brain. This effect was associated with an identical widespread increase in neuronal c-Fos (a cellular proto-onco gene belonging to the immediate early gene family of transcription factors) and a similar stimulation of hippocampal BDNF. Neurons accumulating IGF-I showed increased spontaneous firing and a protracted increase in sensitivity to afferent stimulation. The authors also completed experiments that blocked the uptake of IGF-I by brain cells and concluded that serum IGF-I mediates activational effects of exercise on the brain.

Ding and colleagues (2006) support the hypothesis that IGF-I plays a vital role in the exercise-induced effects on brain function and suggest that the effects of exercise on brain neuronal and cognitive plasticity are in part modulated by a central source of IGF-I. They showed that acquisition of the MWM task was

significantly improved following 5 days of voluntary exercise; specifically on days 3, 4 and 5 the exercise groups were faster at finding the platform than sedentary control rats. Blocking the IGF-I receptor did not have any effect on learning acquisition, however, it fully prevented the exercise-induced improvement in memory retention as assessed by a probe test 2 days after the last MWM training day. The results also revealed that the IGF-I pathway modulates an end product of BDNF action synapsin I and signal transduction cascades downstream of BDNF activation phosphorylated CAMKII and phosphorylated MAPKII confirming a strong link between IGF-I and BDNF in the context of exercise.

This research defines an important role for IGF-I and suggests a positive correlation with BDNF action in mediating the exercise-induced effects within the brain.

1.8 Exercise and Vascular Changes

Regular physical activity may attenuate age-related decreases in arterial wall elasticity and poor endothelial function. Exercise training induces marked vascular remodelling by increasing angiogenesis and arteriogenesis (Kojda & Hambrecht, 2005). These changes are likely associated with functional changes and ultimately improve organ blood flow. Kojda and Hambrecht (2005) investigated the signal transduction pathways involved. They observed that mechanotransduction mechanisms in endothelial and smooth muscle cells that are activated by physical forces, such as shear stress and cyclic stretch, stimulate various signal transduction cascades. Consequently, phosphorylation of kinases including Akt, PI-3K and MAPK ultimately led to the up-regulation of genes mediating anti-atherogenic effects. On the basis of their observations the authors concluded that exercise training may be viewed as an effective antioxidant and anti-atherogenic therapy.

It is possible that exercise-induced vascular changes might contribute to the exercise-induced changes that occur within the brain. Several growth factors are increased in the circulation following exercise including IGF-I and VEGF. The implication that IGF-I is closely involved in mediating the effects of exercise on cognitive function and the confirmation that exogenous peripheral infusion of this

growth factor can stimulate neurogenesis in the absence of exercise (Aberg *et al.*, 2000) provides strong evidence to support a hypothesis that peripheral and central mechanisms may interact to mediate the effects of exercise on the brain.

Fabel and colleagues (2003) investigated whether somatic mechanisms may also be associated with the enhancement in neurogenesis observed as a result of 1 week of voluntary physical exercise. The effects of VEGF on cultured neural stem/progenitor cells isolated from the adult rat hippocampus were evaluated to determine the potential action of VEGF on neurogenesis. The results show that peripheral VEGF is necessary for an exercise-induced increase in neurogenesis and suggest that somatic signalling networks can function independently of the central regulatory networks that are typically considered in the context of hippocampal neurogenesis.

In addition, cerebral angiogenesis has been observed in aged female rats (22 months) following 3 weeks treadmill exercise where rodents exercised for 30min per day (Ding *et al.*, 2006). Specifically, exercise produced an increase in the density of microvessels within the cerebral vasculature of aged rats. These changes were associated with an increased expression of the four isoforms of VEGF mRNA (120, 144, 164, 188) and VEGF protein. Interestingly, Ding and colleagues (2006) more recently demonstrated that exercise pre-conditioning strengthens brain microvascular integrity in a rat stroke model indicating a neuroprotective effect of exercise by way of vascular adaptations to exercise.

1.9 Exercise and Oxidative Stress

Age-related changes within the brain may be caused by free radical reactions (Harman, 1998). A current hypothesis specifically states that oxidative stress may contribute to the age-related impairment in learning and memory (Fukui *et al.*, 2002). Both during the normal aging process and in neurodegenerative disorders reactive oxygen species (ROS) and reactive nitrogen species (RNS) increase to levels that result in a toxic oxidative shift in the redox state of the central nervous system. An increase in the level of ROS and RNS is thought to arise from both an increase in production and a reduction in the activity of antioxidant protective mechanisms (Dirksen, 2002). As a result the aged brain is increasingly susceptible to oxidative stress. Given that oxidative stress is increased with age it is plausible

that an antioxidant effect of exercise may be involved in conferring neuroprotection.

Physical activity evokes an increased production of oxidative species, depending mainly on the intensity of the muscle actions (Apor & Radi, 2006). Although an immediate response of the body to exercise is an increase in the generation of ROS and RNS it has been suggested that regular exercise, by causing adaptation, could decrease the incidence of ROS and RNS-associated diseases. In line with this theory it has been shown that the elderly who are physically active may benefit from an exercise-induced adaptation in cellular antioxidant defense systems (Ji, 2002). Indeed, adaptations to exercise that may decrease oxidative stress include increased antioxidant defenses, reduced basal production of oxidants and reduction of free radical leak during oxidative phosphorylation (Leeuwenburgh & Heinecke, 2001).

Radak and colleagues (2001) revealed that regular exercise, alongside improving cognitive function, decreased oxidative damage in the rat brain. 4-week-old and 14-month-old male Wistar rats were randomly assigned to swimming and sedentary groups. These data collectively revealed that swimming training for 9 weeks improved some cognitive functions in rats, with parallel attenuation of oxidatively damaged proteins. The authors speculated that an increase in the activity of antioxidant enzymes within the brain was most probably due to the excess formation of free radicals in response to regular physical exercise.

1.10 Forced Exercise and Hippocampal Function

It has been suggested that forced exercise, because of the associated stress, may not be as successful as voluntary exercise at inducing positive effects on brain function. Moraska and colleagues (2000) cited that treadmill running produces negative physiological adaptations that are indicative of chronic stress and has the potential to impact negatively on measures of interest. The adrenal steroid corticosterone, a principal glucocorticoid associated with the stress response, has profound effects on the hippocampus and it is plausible that it may act to modulate memory formation. It is presumed that the level of circulating corticosterone would be elevated to a greater extent following forced exercise than following voluntary exercise. It may be speculated that forced exercise better

simulates aspects of human exercising behaviour. To be more explicit, most forms of exercise undertaken by humans involve an element of stress i.e. competitive sports. In light of this, it seems equally important to identify the potential for forced exercise to improve learning and memory.

Ang and colleagues (2006) observed improvements in spatial learning with a forced exercise regime using a modified version of the MWM task. Young rats that exercised for 12 weeks had a reduced time required for spatial acquisition and a superior probe trial performance compared with age-matched control animals. Albeck and colleagues (2006) also investigated whether mild forced treadmill exercise over 7 weeks could improve spatial learning in aged Brown Norway/Fisher rats (23 months). The results showed an exercise-induced enhancement in spatial learning as assessed by the MWM.

Kim and colleagues (2002) investigated the effects of forced treadmill running on cell proliferation and apoptosis in the dentate gyrus, one of the few brain regions where neurogenesis occurs. Sprague-Dawley rats (5 weeks) ran on 7 consecutive days for 30min per day on a motorised treadmill. To ensure that forced running was the only physiological stress, electric shocks were not used to force the animals to run. The authors found that treadmill exercise increased cell proliferation without altering apoptosis in the dentate gyrus. This group published another study in 2004 that confirmed treadmill exercise has the potential to increase cell proliferation in the dentate gyrus. In this study adult Sprague-Dawley rats of different ages (4-week-old, 8-week-old and 62-week-old rats) were exercised for 30min once a day for 5 consecutive days. Treadmill exercise increased cell proliferation in all age groups. However, the most potent enhancing effect was observed in the 8-week-old rats suggesting age is an important factor in the regulation of cell proliferation in the dentate gyrus.

Although not directly associated with exercise an interesting review completed by Schaff and colleagues (2000) proposed a mechanism of action for corticosterone suppression of BDNF. It was suggested that activated mineralocorticoid and glucocorticoid receptors repress transcriptional activity of the BDNF promoter (site-specifically) through interaction with other transcription factors. In relation to learning the authors stated that a rise in corticosterone levels during water maze training does not suppress BDNF expression in any hippocampal subfield.

Additionally, high BDNF expression levels in specific subfields, dentate gyrus, CA1 and CA3, were correlated with good memory performance. Given that BDNF has been targeted as a key mediator of the positive effects of exercise on brain function it may be speculated that a rise in corticosterone levels may not have significant implications for the effects of exercise on cognition.

In line with this, Radeki and colleagues (2005) observed that BDNF protects against stress-induced impairments in LTP and spatial learning and memory. Chronic immobilization stress (2hr/day for 7 days) significantly elevated plasma corticosterone, impaired spatial learning and attenuated LTP in male Long-Evans rats. BDNF was infused into the left hippocampus in a group of rats for 14 days beginning 7 days prior to the immobilization stress. BDNF protected against the deleterious effects of stress and despite the presence of elevated corticosterone BDNF-infused rats performed at a similar level to control animals in a spatial learning task. This study suggests that there is the potential for BDNF to override the effects of corticosterone on hippocampal function thus counteracting the negative effects of stress.

To bring the focus back to exercise, Adlard and Cotman (2004) subjected a group of young mice to a 2hr immobilization stress that effectively elevated circulating corticosterone. This stress program was associated with a reduction in BDNF protein within the hippocampus at 5 and 10hr post stress that returned to baseline at 24hr. To identify whether exercise may affect this stress-related reduction in BDNF a group of animals were given voluntary access to running wheels for 3 weeks prior to the stress. The results demonstrated that exercise may overcome the stress-induced effects of corticosterone on BDNF protein.

Collectively, this research confirms that forced exercise, in addition to voluntary exercise, may improve neurogenesis, synaptic plasticity and learning and memory. The literature also provides some insight into the mechanisms by which forced exercise may improve cognitive function.

1.11 Variability in Studies Investigating the Effects of Exercise on Cognition

Although there is definite agreement that exercise positively affects cognition there is some inconsistency in the literature, particularly in relation to the underlying mechanisms involved. Additionally, it has been speculated that

different factors may affect the magnitude of an exercise-induced effect on the brain. An explanation for the inconsistencies observed could be the large variety of study designs used. A variety of exercise programs, animal populations, animal species and types of cognitive assessment have been used to assess the effects of exercise on brain function. By focusing singly on exercise programs, both voluntary and forced paradigms, different intensities and different durations of exercise have been studied. This one example highlights that the differences in study design are vast and may account for conflicting results. Interestingly, little research has explored the extent to which these variations may effect the exercise-induced changes within the brain.

Kim and colleagues (2003) studied the effects of a variety of exercise programs on cell proliferation. The authors specifically investigated whether cell proliferation was dependent on the magnitude of exercise; light, moderate and severe exercise groups were defined. The effect of exercise duration was also explored; animals exercised for 1, 3, 7, 14 or 28 days. The results showed that cell proliferation in the dentate gyrus of rats is modulated by the intensity and duration of exercise. Cell proliferation was most prominent in the light-exercise group and reached a maximum level after 7 days of exercise.

An obvious comparison between study designs may be an investigation of the differential effects of acute and chronic exercise programs on learning and memory and associated cellular modifications. Surprisingly, this has rarely been addressed. The vast range of exercise program duration explored extends from a 2 day exercise exposure to 9 week programs and longer. Molteni and colleagues (2002) specifically investigated the differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus. Young adult male Sprague-Dawley rats were given access to a voluntary running wheel for 3, 7 and 28 days; only rats that ran a minimum of 5km per night were included in the study. Following exercise an analysis of the expression of hippocampal genes was completed. The BDNF gene was up-regulated at all time points as were genes associated with the glutamatergic system. The largest exercise-induced up-regulation was observed in genes involved in synaptic trafficking, signal transduction pathways and transcription regulators. Synapsin I was predominantly upregulated with short periods of exercise (3 and 7 days), consistent with its role

in synaptic vesicle release. Synaptotagmin expression progressively increased with longer periods of exercise, consistent with its role in synaptic vesicle formation. Members of the CAMK family showed the largest increase with short periods of exercise where members of the MAPK pathway showed a progressive increase with a longer duration of exercise (after 7 days). The largest increase in CREB mRNA was observed after 7 days exercise; this is consistent with the induction of MAPK members. Collectively, these results suggest a definite differential effect of acute and chronic exercise on plasticity-related hippocampal genes. To support the theory that exercise duration is relevant to the exercise-induced effects on the brain a recent review by Cotman and colleagues (2007) stated that although some studies show improvements in cognitive function after 1 week of exercise most benefits have been associated with long-term exercise.

Ra and colleagues (2002) demonstrated that treadmill running and swimming increase cell proliferation in the rat dentate gyrus. Additionally, this group assessed whether the effects of running and swimming on cell proliferation were intensity-dependent. Sprague-Dawley rats in the running group exercised for 30min each day, those in the swimming group swam for 5min each day. Exercise sessions were completed over 3 consecutive days. The increase in cell proliferation associated with exercise was observed most potently in light intensity and 5min swimming groups. This result supports the contention that the specific effects of exercise on the brain are dependent on the intensity of exercise.

Johnson and Mitchell (2003) tested a hypothesis that exercise-induced changes in hippocampal BDNF and NT3 differ among rat strains engaging in voluntary wheel running (1 or 7 nights). Four strains of 7/8-week-old rats were used in the study (Sprague-Dawley, Brown Norway, Dark Agouti and PVG). When data from all strains were combined hippocampal BDNF levels were seen to increase with 7 nights of wheel running and were positively correlated with the distance run during the previous night. Interestingly, exercise decreased NT3 levels and NT3 levels were negatively correlated with distance run the previous night. In relation to possible differences in the effects of exercise on rat strain the authors conclude that genetic background influences hippocampal BDNF but not NT3 levels. In light of this, it was suggested that strain differences should be accounted for in the design of future experiments.

Considering these studies collectively, it is clear that caution must be exercised in assessing the reported effects of exercise on cognitive function and the mechanisms mediating these effects; while there may be some generic effects of exercise on the brain, some effects are likely to be specific to rat strain, exercise intensity, exercise duration and other factors.

1.12 Exercise and Cognitive Function in Aged Animals

A wealth of literature exists to support the benefits of exercise on cognitive function in young animals. Given that the drive of this research is primarily derived from an expanding elderly population and the increasing prevalence of neurodegenerative disease it seems especially relevant that research explores how exercise impacts brain function in an elderly population. The exercise-induced effects on cognition may be dependent on a number of factors. Some of these have been discussed in the previous section. Thus, it seems plausible that the exercise-induced effects on cognitive function may also be age-specific.

Some research has suggested that there is no differential effect of age on the potential for exercise to enhance brain function. A study that has previously been mentioned was that of van Praag and colleagues (2005) where they demonstrated an increase in learning and neurogenesis with voluntary wheel running in aged mice that were sedentary until 19 months of age. Young (3-month-old) and old (19-month-old) Sprague-Dawley mice were assigned to sedentary and exercising groups. Runners had unlimited access to a running wheel for 45 days. Exercise enhanced acquisition of the hidden platform task in the MWM compared with age-matched sedentary controls. The authors speculated that an earlier onset of running in the aged group may have maintained cognitive function to an even greater extent. Interestingly, although earlier research by van Praag and colleagues (1999b) produced an increase in spatial learning following exercise, no significant effect of exercise on learning acquisition was observed in young animals in this study. In aged runners cell survival returned to the level of young controls. The results of the study also revealed that there was no exercise-induced angiogenesis in aged animals while exercise did enhance perimeter and surface area of blood vessels in young animals. Despite this result the lack of exercise-induced angiogenesis does not appear to be a rate-limiting factor for neurogenesis given

the significant increase in new neurons in aged runners. In summary, this study demonstrates that exercise does have the potential to restore spatial learning and neurogenesis in aged mice.

Radak and colleagues (2001) studied the effects of exercise on cognitive function and oxidative damage in young (4 week old) and middle aged (14 month old) Wistar rats. Again, this study has been discussed previously but not specifically in respect of age differences. Exercising rats engaged in a swimming regime for 9 weeks (1 hr/day, 5 days a week). The age- associated decline in performance of the passive avoidance test, representing both short and long-term memory, was prevented by exercise but no significant exercise-induced enhancement in this type of learning was observed in the young animals. This result might suggest that although exercise offers neuroprotection against an impairment in cognitive function it does not always act as a cognitive enhancer when no cognitive deficit is present. Performance on the pole-jumping active avoidance test was significantly improved in both exercise groups compared with age-matched controls. The results also reveal that exercise training in both age groups reduced the accumulation of reactive carbonyl derivatives. These results suggest that the impact of exercise on cognitive function and oxidative damage within the rat brain is not markedly affected by age. Exercise was shown to improve some forms of cognitive function in both age groups and also reduced oxidative damage in the rat brain in both age groups. The particularly interesting result is that when an age-related cognitive impairment in the passive avoidance response exists exercise can offer neuroprotection to ameliorate this decline in cognitive function but when there is no evidence of cognitive impairment exercise does not always produce a significant cognitive enhancement.

Other studies have also lent support to the argument that there is the potential for exercise to positively impact cognitive function in aged animals. Albeck and colleagues (2006) reported an enhancement in spatial learning in aged rats following 7 weeks of mild forced treadmill exercise and Nichol and colleagues (2007) reported that 3 weeks of voluntary wheel exposure improved cognitive performance, assessed by the radial arm maze, in the aged mouse. However, there is some inconsistency. Barnes and colleagues (1991) did not see an enhancement in spatial learning in aged rats following a 10 week exercise program using a

motorised treadmill. Additionally, Hansalik and colleagues (2006) investigated the effects of voluntary and forced exercise on the age-related impairment in MWM performance and did not report any positive effect of exercise on this from of learning.

It must also be considered that the magnitude of an exercise-induced effect may depend on age. Kim and colleagues (2004) explored the effect of treadmill exercise on cell proliferation in the dentate gyrus of 4 week old, 8 week old and 62 week old male Sprague-Dawley rats. Exercising animals engaged in treadmill running for 30min once a day for 5 consecutive days. Cell proliferation in the dentate gyrus was evident in all exercising groups. The most prominent cell proliferation was observed in 4 week old rats and the rate of cell proliferation declined with increasing age. A result that is of particular interest is that the most potent enhancing effect of treadmill exercise on cell proliferation appeared in the 8 week-old group. This demonstrates that the enhancing effect of treadmill exercise on cell proliferation is dependent on age.

Similarly, Adlard and colleagues (2005) investigated whether the exercise-induced expression of BDNF within the hippocampus varies across life span. The induction of BDNF protein in the hippocampus of young (2 months), late middle-aged (15 months) and aged (24 months) animals was examined over 4 weeks of voluntary exercise. Male C57 B1/6 mice were allowed to exercise for 1, 2, 3 or 4 weeks. Average running distances decreased with age and where aged animals maintained a constant level of activity over time the younger groups increased their average running distance over time. All animals demonstrated a biphasic profile of BDNF induction; a significant increase was seen after 1 week of exercise and decreased to near baseline sedentary levels at 2 weeks. Following this BDNF protein levels were increased significantly compared to baseline, but this was most significant in the young animals. The key results of this study are twofold. Firstly it highlights that the effects of exercise on the brain may be dependent on duration of exercise. Secondly and most pertinent to this discussion of the literature, it demonstrates that while animals in all age groups were responsive to exercise as measured by the induction of BDNF protein, the most striking exercise-effect was seen in young animals.

Despite the inconsistencies observed it is very promising that aged populations of

animals may have the potential to benefit from exercise and that exercise-induced cognitive enhancements have been demonstrated. Perhaps the magnitude of the effects of exercise on hippocampal neurogenesis or hippocampal BDNF induction is not as great in aged populations but the fact that there is evidence demonstrating the potential for functional improvements in an older age group is encouraging nonetheless.

1.13 Environmental Enrichment and Cognitive Function

Any novel stimulus such as natural or artificial objects, scents and new foods are considered enriching. In scientific research, environmental enrichment is provided by environmental stimuli that will improve quality of life, provide stimulation and increase physical activity. Environment may also be enriched by the presence of other animals. Similar to exercise, environmental enrichment has been associated with enhanced learning. Interestingly, Lambert and colleagues (2005) proposed that different elements of an enriched environment have markedly distinct effects on spatial memory and synaptic alterations. The contribution of cognitive stimulation, exercise and acrobatic training to improved memory and synaptic plasticity was investigated. Exercise was the only intervention that improved spatial working memory, despite the fact that both exercise and cognitive stimulation increased synaptophysin levels in the neocortex and hippocampus.

Studies investigating the effects of environmental enrichment have been completed on young and aged animals. Duffy and colleagues (2001) indicated that exposure of young mice to environmental enrichment altars the PKA-dependence of LTP and enhances one type of hippocampal-dependent memory. In addition, Artola and colleagues (2006) demonstrated an enhancement in LTP and long-term depression (LTD) following 3-5 weeks of environmental enrichment in adult rats (300-350g) thus providing evidence that enrichment enhances synaptic plasticity. Given that environmental enrichment reduced paired-pulse facilitation, the authors suggest that an increase in pre-synaptic release facilitating the induction of LTP and LTD may contribute to the enhancement in synaptic plasticity observed. Paylor and colleagues (1992) also observed an enhancement in MWM performance in young rats following brief exposure to an enriched environment. Improvements in learning were seen in young animals exposed to an enriched

environment for 12 days but not in a group of age-matched animals exposed to an enriched environment for 6 days.

Considering the impact of environmental enrichment in aged animals, Frick and colleagues (2003) suggested that environmental enrichment initiated at middle age can reduce age-related impairments in spatial memory in mice. Additionally, environmental enrichment has been associated with an enhancement in hippocampal neurogenesis in aged rats that has been linked with an improvement in spatial learning (Nilsson *et al.*, 1999). Escorihuela and colleagues (1995) demonstrated that 6 months environmental enrichment (considered long-term environmental enrichment) could prevent the cognitive loss associated with aging. The study also investigated the effect of rat strain (hypoemotional Roman high-avoidance and hyperemotional Roman low-avoidance rats were used) on the potential for environmental enrichment to improve learning. Although strain appeared to have an effect on MWM performance in control animals, there was no effect of rat line on the ability of environmental enrichment to improve learning in aged rodents.

An interesting study completed by Harburger and colleagues (2007) investigated the age-dependent effects of environmental enrichment on spatial reference memory in male mice. The results revealed that 24hr/day environmental enrichment for approximately 6 weeks significantly improved spatial memory in the MWM in aged males, but not in young or middle-aged groups, suggesting that aged animals are most responsive to the effects of environmental enrichment.

Collectively this research suggests that the positive effects produced by environmental enrichment on cognitive function are in some instances age-specific and task-specific.

1.13.1 Environmental Enrichment and Cognitive Function; Mechanism of Action

It has been frequently shown that hippocampal neurogenesis accompanies environment enrichment-induced improvement in learning. Segovia and colleagues (2006) demonstrated an increase in neurogenesis in the dentate gyrus of young and old rats housed in an enriched environment for 8 weeks. Results that were specific to aged rats showed that enriched housing in addition to increasing

neurogenesis, also increased glutamate and γ-aminobutyric acid (GABA) levels in the CA3 area of the hippocampus. The authors speculate that changes in glutamate and GABA levels in the CA3 region of the hippocampus could be induced by a modification of the circuitry of this subfield in parallel with the extension of axons of new neurons. Additionally, they propose that the increases in glutamate and GABA observed in response to environmental enrichment occur due to changes in the neuron-astrocyte network; it has been reported that the number of astrocytes and their activity is altered with age (David *et al.*, 1997; Vasquez *et al.*, 1992).

The results of a study completed by Nilsson and colleagues (1999) have already been mentioned but it is relevant here to highlight that this group specifically linked an enhancement in hippocampal neurogenesis with improved spatial learning to environmental enrichment in an aged rodent population. Kempermann and colleagues (2002) also observed that adult hippocampal neurogenesis was fivefold times higher in mice living in an enriched environment from the age of 10 to 20 months when compared with age-matched controls. Interestingly, Meshi and colleagues (2006) investigated a causal link between the effect of environmental enrichment on neurogenesis and its effect on spatial learning. Although an increase in hippocampal neurogenesis was observed with adult mice housed in an enriched environment, when adult hippocampal neurogenesis was blocked, spatial learning was still improved following environmental enrichment. This result suggests that (a) environmental enrichment may alter hippocampal-dependent behaviour in mice regardless of neurogenic capability and (b) that newborn cells do not mediate the effects of enrichment on this specific population.

Other cellular changes that have been associated with an environment enrichment-induced improvement in spatial learning include an up-regulation of growth factors such as NGF and NT3 in the hippocampus (Torasdotter *et al.*, 1998). Rossi and colleagues (2006) reported a role for neurotrophins in mediating the effects of environmental enrichment on hippocampal function. This study specifically focused on the role of BDNF and NT4 in mediating the increase in hippocampal neurogenesis associated with environmental enrichment. The results of the study led the authors to conclude that BDNF but not NT4 is required for

environmental induction of neurogenesis. Interestingly, when environmental enrichment has been seen to attenuate cognitive deficits caused by brain injury the improvements in learning may not consistently be correlated with an increase in neurotrophin expression. Hicks and colleagues (2002) induced ischemic brain injury by lateral fluid percussion and, while environmental enrichment attenuated the experimentally-induced learning deficits, no changes in BNDF, TrkB or NT3 hippocampal gene expression were observed.

An improvement in MWM performance following 12 days exposure to an enriched environment was associated with an increase in hippocampal protein kinase C (PKC) activity in a study completed by Paylor and colleagues (1992). PKC is thought to be a neural substrate underlying learning and memory processes. The authors propose that a functional relationship exists between spatial learning and hippocampal PKC activity.

It seems that further research is required to more clearly elucidate the mechanisms underlying the improvements in learning and memory induced by environmental enrichment.

1.13.2 A Comparison of Exercise and Environmental Enrichment

It is clear that both exercise and environmental enrichment have the potential to positively influence cognitive function but these interventions may not produce the same magnitude of effect within the brain and it may be the case that the mechanisms involved in mediating these effects on cognitive function are different.

Gobbo and O'Mara (2005) demonstrated that exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration. Kainic acid induces excitotoxicity by overstimulating glutamate receptors and causing neuronal death by necrosis and apoptosis. Exercise prior to kainic acid-induced brain damage was shown improve spatial learning, assessed by the MWM and object exploration. This effect of exercise on hippocampal-dependent learning was associated with an increase in BDNF. The authors state that, despite a marked increase in BDNF in the dentate gyrus, neither exercise nor environmental enrichment conferred protection against neuron loss in CA1, CA2 and CA3 areas of the hippocampus induced by the kainic acid model

of neuronal injury. This would suggest the exercise-induced recovery of spatial learning was mediated by changes in other brain regions.

Van Praag and colleagues (1999a) investigated the impact of various components of environmental enrichment on neurogenesis in the dentate gyrus. Mice were assigned to various conditions including water-maze learning (learning group), swim-time-yoked control (swimming group), voluntary wheel running (running group), enriched housing (enriched group) and standard housing (control group). The results showed that voluntary wheel running and enriched housing stimulated an increase in the number of surviving newborn cells; the magnitude of this effect was similar between groups. Despite this similarity Olson and colleagues (2006) suggest that environmental enrichment and voluntary exercise should be considered distinct interventions with regard to hippocampal plasticity and associated behaviours. Although the authors recognize an equivalent effect of environmental enrichment and voluntary exercise on increasing hippocampal neurogenesis and improving spatial memory they also believe that the two manipulations affect different phases of the neurogenic process in different ways. It is suggested that voluntary exercise induces cell proliferation by the convergence of somatic and cerebral factors in the dentate gyrus where environmental enrichment induces cell survival in the dentate gyrus by means of cortical restructuring. Thus, the different interventions may significantly enhance neurogenesis via dissociable pathways.

1.14 Summary

It is clear that both basic animal models and clinical data overwhelmingly support the theory that exercise intervention is a major protective factor against neurodegeneration of varied etiologies (Kiraly & Kiraly, 2005). Additionally, exercise-induced improvements in cognitive function have been observed in young animals. This suggests that exercise may, under some circumstances, have the ability to enhance hippocampal function as well as confer neuroprotection against neurodegenerative decline or brain insult.

It is well-recognized that neurotrophins, most prominently BDNF, are central to the exercise-induced effects on brain function. Although this area of research has been extensively studied, the precise mechanisms that underlie an exercise-

induced improvement in cognitive function have yet to be identified. Numerous mechanisms have been explored and some theories are well-supported. However, the huge variability in study design makes it increasingly difficult to find consistent agreement in the literature.

In addition, there are areas within this umbrella of research that have received little attention and perhaps deserve more investigation. Specific comparisons of acute and chronic exercise programs and the differential effects of age on the exercise-induced effects on cognitive function are not well-explored.

1.15 Research Aims

The main aim of this research is to investigate the effects of exercise on hippocampal function in young, middle-aged and aged rodent populations. Moreover, a comparison of the effects of acute and chronic exercise programs on cognitive function within these differently aged populations will be completed. Finally, the molecular mechanisms involved in mediating these changes will be explored.

Chapter 2

Materials and Methods

Chapter 2

2.1 Materials

Animals

Male Wistar rats (3-4months old)	Bioresources, TCD
Male Hans Wistar rats (13-15 & 22-23 months old)	Charles River
Laboratories UK	

ELISA

Maxisorp immunoplates for ELISA	Nunc
Human BDNF ELISA kit (Duoset)	R&D Systems
Human BDNF Emax ImmunoAssay System	Promega
Rat IL-1β kit (Duoset)	R&D Systems

Equipment

Exer 3/6 treadmill	Columbus Instruments

Laboratory Chemicals

Acrylamide	Sigma
Ammonium Persulphate	Sigma
Anti-IgY horseradish peroxidase-conjugated antibody	Promega
Bio-Rad dye reagent concentrate	Bio-Rad
Bis-acrylamide	Sigma
Block and Sample 5X Buffer	Promega
Bovine serum albumin	Sigma
Bromophenol blue	Sigma
Calcium chloride ($CaCl_2$)	Lennox
Chemiluminesence Hyperfilm	Amersham
Dimethyl sulphoxide	Sigma
Ethanol	Lennox
Filter paper No.1, No.3	Whatman
Glucose	Lennox

Glycerol	Sigma
Glycine	Sigma
Hydrochloric acid	Lennox
Hydrogen peroxide	Sigma
Magnesium Chloride ($MgCl_2$)	Sigma
Magnesium sulphate	Sigma
β-Mercaptoethanol	Sigma
Methanol (MeOH)	Fluka
Nitrocellulose membrane	Amersham
Non-fat dry milk	Marvel
Potassium chloride (KCl)	Sigma
Potassium phosphate (KH_2PO_4)	Sigma
2-propanol	Sigma
ReBlot Plus strong antibody stripping solution	Chemicon
Sodium carbonate (Na_2CO_3)	Sigma
Sodium chloride (NaCl)	Sigma
Sodium dodecylsulphate (SDS) 99%	Sigma
Sodium hydrogen carbonate ($NaHCO_3$)	Lennox
Sodium hydroxide (NaOH)	Sigma
Sodium phosphate, monobasic (NaH_2PO_4)	Sigma
Sodium phosphate, dibasic	Sigma
Streptavidin-HRP conjugate (Anti-IgY)	Promega
Sucrose	Sigma
Sulphuric Acid (H_2SO_4) 98%	Sigma
Tetramethylethylene-diamine (TEMED)	Sigma
Tetramethylbenzidine (TMB)	R&D Systems
TMB One solution	Promega
Tris-base	Sigma
Tris-HCL	Sigma
Tween-20	Sigma
Urethane	Sigma

Laboratory Plastics

Pipette tips	Starstedt
Microtubes (1.5ml, 0.5ml)	Starstedt
Plastic transfer pipettes (3ml, 1ml)	Starstedt
Laboratory film	Parafilm

Molecular Reagents

Absolute ethanol	Sigma
Agarose	Invitrogen
Biosphere filter tips (1000, 200, 100 μl)	Starstedt
Ethidium bromide	Sigma
High capacity cDNA archive kit	Applied Biosystems
Loading dye (6x)	Promega
Molecular grade water	Sigma
Optical adhesive covers	Applied Biosystems
PCR tubes	Starstedt
RNA*later*™	Ambion
RNase away	Invitrogen
RNase-free microfuge tubes (2ml, 1.5ml)	Ambion
RNAse Zap wipes	Ambion
Total RNA isolation kit	Macherney-Nagel
Taqman gene expression assays (Table 2.1)	Applied Biosystems
Taqman universal PCR master mix	Applied Biosystems
10x TBE buffer	Invitrogen
96-well optical reaction plates	Applied Biosystems

Western Immunoblotting Reagents and Antibodies

Anti-mouse IgG	Sigma
Anti-rabbit IgG	Sigma
Filer paper No.3	Whatman
Molecular weight marker	
• Dual Colour, precision plus protein standards	Biorad
Monoclonal anti-βactin antibody	Sigma

Monoclonal anti-ERK2 antibody	Santa Cruz
Monoclonal anti-phospho ERK antibody	Santa Cruz
Mouse recombinant proBDNF antibody	Alamone
Nitrocellulose membrane	Sigma
Polyclonal anti-p75 antibody	Dr P Barker, McGill University
Polyclonal anti-TrkB antibody	Upstate
Reblot Plus Strong Antibody Stripping Solution	Chemicon
SuperSignal	Pierce

2.2 Animals

Male rats were used for all experiments. Young rats (4 months) were the Wistar strain, middle-aged (13-15 months) and aged (22-23 months) rats were the Hans Wistar strain. Using different strains of rats was unavoidable due to the limited availability of suppliers of aged rodents. Although there is some evidence to suggest that exercise-induced changes within the hippocampus are influenced by genetic background, no differences between Wistar and Hans-Wistar rats have been published. Young animals were housed in standard cages (4 animals per cage) and aged animals were housed in large cages (3/4 animals per cage). Animals were provided with food and water *ad libitum* and experienced a 12:12hr light-dark cycle in a temperature-controlled environment (20-22°C). All animals were acclimatised to their new surroundings prior to experiments (7 days acclimatisation was standard for aged animals). Acclimatisation allowed animals to habituate to their new surroundings prior to experiments and become familiar with handling. Experiments were conducted under national law and European union directives on animal experiments.

2.3 Weight assessment

Animals used for the chronic exercise studies were weighed at the beginning of the study and at regular intervals throughout the experiment to give an indication of their general health.

2.4 Exercise program

All animals were familiarized to the motorised treadmills (Exer 3/6 treadmill, Columbus Instruments) and assigned to control and exercising groups of equal number. A picture of one of the animal treadmills is shown in Figure 2.1. Familiarisation involved exposure to the running treadmill for 15-30min on two consecutive days unless otherwise specified. The speed of the treadmill belt was gradually increased from 10m/min to 12.5-17m/min during acclimatisation; maximum speed was dependant on the age of the animals.

Figure 2.1 The Exer 3/6 animal treadmill, Columbus Instruments

The motorised animal treadmill used to exercise animals is shown. Clear perspex dividers were used to separate the treadmill belt into 2 or 3 lanes.

The running behaviour of the animals was observed during the familiarisation period and keenness to run was assessed. Following familiarisation to the treadmills animals were divided equally into control and exercising groups; those that ran consistently on the treadmills were selected for the exercising groups.
The acute exercise program consisted of 7 days consecutive running at a speed of 17m/min for 60min (approximately 1km per day). Aged animals ran at a speed of 12.5m/min for 4 x 20min sessions with a rest-interval of 10min. The chronic exercise program consisted of 3 exercise sessions per week for 8 months; during the first week of exercise the duration and speed of exercise was increased from 2 x 30min sessions at 15m/min to 2 x 40min sessions running 17m/min. All animals exercised for approximately 1km per day whether participating in the acute or chronic studies. Although the exact distance run by each rat was not recorded, it was believed that the exercise programs chosen would ensure that rodents ran for distances greater than the minimum distance required to induce changes in cognition. Previous research suggests that young rats exercising for a minimum of 100m per night for 4 consecutive nights is sufficient to induce an increase in BDNF in the dentate gyrus and recover experimentally induced deficits in spatial learning (Shaw *et al.*, 2003).
At one end of the treadmill belt there are wire loops through which a mild electric

shock can be delivered; these act to motivate the rats to run continuously and were activated at low levels throughout all exercise sessions. Rats were observed while exercising to ensure they ran continuously and to monitor for signs of undue stress. Occasionally rats displayed behaviour that suggested they may be distressed, such as hyperactivity and squeaking, in this situation rats were immediately removed from the treadmill and if necessary housed individually for 24hr. All control rats were placed on stationary treadmills for the same duration as exercising animals.

2.5 Assessment of LTP

Assessment of LTP was completed at the end of the exercising period. For acute exercise studies, LTP experiments were performed on exercising and control animals the day after the exercise period was completed. LTP experiments were conducted during the week that followed cessation of exercise for the chronic exercise studies. Rats were anaesthetized with urethane (1.5g/kg intraperitoneal injection) prior to LTP assessment. Initial injections of 1g/kg were administered and topped up as necessary. Deep anaesthesia was indicated by lack of pedal reflex. Fur was removed from the scalp of the animal and the head was positioned in a stereotaxic frame. A scalpel was used to make a midline incision along the centre of the scalp and the skin was held back with metal clips. To allow clear identification of lambda and bregma (skull markings) the periosteum was scraped clean. A dental drill was used to remove part of the skull and expose the brain and dura mater. The dura mater was pierced and stripped away to allow implantation of the electrodes.

The stereotaxic unit which comprised the recording chamber was attached to a laboratory bench and protected by a Faraday cage to ensure that there was minimal intereference from the external environment. All instrumentation was grounded to eliminate for 50Hz cycle noise.

2.5.1 Implantation of electrodes

A bipolar stimulating electrode was positioned 4.4mm lateral to lambda and a unipolar recording electrode was positioned 2.5mm lateral and 3.9mm posterior to bregma. The electrodes (Clark Electromedical, UK) were initially placed on the

surface of the brain and then gradually lowered 2mm into the brain. The electrodes were slowly moved further into the brain passing through the cortical and hippocampal layers to the perforant path (bipolar stimulating electrode) and the granule cell layer of the dentate gyrus (unipolar recording electrode). Throughout this process, the position of the electrodes was closely monitored by generating a regular pulse through the stimulating electrode (0.1msecs duration, 2msecs delay, 4V amplitude, 0.1Hz frequency). The recording electrode detected the response and displayed this information on an Apple Macintosh computer (Performa 200). The electrodes were adjusted until the characteristic perforant path granule cell synapse response was observed. The end depth of the electrodes was 2.5-3.5mm and 2.5-3mm for the recording and stimulating respectively. The intensity of the stimulus delivered through the stimulating electrode sometimes required adjustment to induce the characteristic EPSP response. The intensity was similar for all animals; mean values (± SEM) were 6.1 ± 0.4, 6.4 ± 0.4 and 5.5 ± 0.2 mV for young, middle-aged and aged animals respectively.

A single square wave of current generated by a constant isolation unit (Isoflex, UK) was delivered at low frequency (0.033Hz, 0.1secs, 2msecs delay) to the bipolar stimulating electrode. The population EPSP response that was evoked was then transmitted through a pre-amplifier (DAM %); Differential Amplifier; gain 75, World Precision Instruments, USA) to an analogue-digital converter (Maclab/2e, Analog Digital Instruments). This digitised system was linked to an Apple Macintosh computer (Performa 200) via the software package Scope, Version 3.36, customised to enable fine control over the generation of the square wave pulses and recording of the response.

Once the characteristic response was obtained, stimuli were delivered at 30secs intervals for 15min to record a baseline response. Following this, 3 high frequency trains of stimuli (250Hz for 200msecs at an intertrain interval of 30secs) were delivered through the bipolar stimulating electrode at 30secs intervals. Test shock recording was continued for a further 45min following high frequency stimulation (HFS). This duration of recording post tetanus is similar to that used by van Praag and colleagues (1999b) and Farmer and colleagues (2004) where recordings were continued for 45min after LTP induction. The EPSPs were displayed continously throughout the experiment and all traces was saved for

analysis.

2.5.2 Analysis of LTP

The degree of LTP may be assessed by analysing the EPSP slope, which gives an indication of excitatory synaptic transmission, and the population spike, which gives an indication of the number of cells discharging action potentials in response to a stimulus. It has been suggested that there is an EPSP/spike disocciation and that LTP is composed of 2 independent components, a synaptic componenet and an EPSP-to-spike coupling component (Taube & Schwartzkroin, 1988). Previous studies investigating the effects of exercise on LTP have shown an increase in EPSP slope following the induction of LTP (Farmer *et al.*, 2004). For these experiments the slope of the initial rising phase of the response was calculated (field EPSP); this gives an indication of excitatory synaptic transmission in the dentate gyrus. All field EPSPs were normalised to baseline (average EPSP for the 5min preceeding HFS) and expressed as a percentage.

2.6 Assessment of Recognition Memory

The object recognition task was completed by young animals following the acute exercise program. Aged animals were not assessed for recognition memory as it was presumed older rodents would not explore their environment without an incentive to do so. Although there is a spatial element to this task it was primarily used to assess recognition memory. The apparatus consisted of a black circular arena (diameter 0.9m, height 0.48m) placed in a dimly-lit room. On day 5 of the acute exercise program rats were familiarised to the arena. Familiarisation was completed on the last 3 days of the exercise program and involved exposure to the empty arena for 15min. Animals initially explored the arena in pairs and finally in isolation. Testing began on day 8, the day after the acute exercise program was complete. Two objects made from lego blocks were constructed and positioned in the arena on day 1; object A and object B.

Figure 2.2 Schematic of the 2-day object recognition task

The schematic shows the object recognition arena on day 1 and day 2. On day 2 of the task a novel object (object C) was positioned in the arena instead of object B.

Animals were placed into the arena for three 5min trials with an inter-trial rest period of 5min. They entered the arena at random entry points and an examiner recorded the time (secs) spent exploring each object using stop watches. Objects were thoroughly cleaned between trials to ensure the absence of olfactory cues. On day 2 of the task one of the objects was randomly exchanged for a novel object (object C). Rats were reintroduced into the open field for a single 5min trial and the time spent exploring each object was recorded. Figure 2.2 is a schematic that shows the object recognition arena and the positioning of the objects A, B and C on day 1 and 2 of the task. To assess performance in the task the time spent exploring each object was expressed as a percentage of the total exploration time.

2.7 Assessment of Spatial Learning

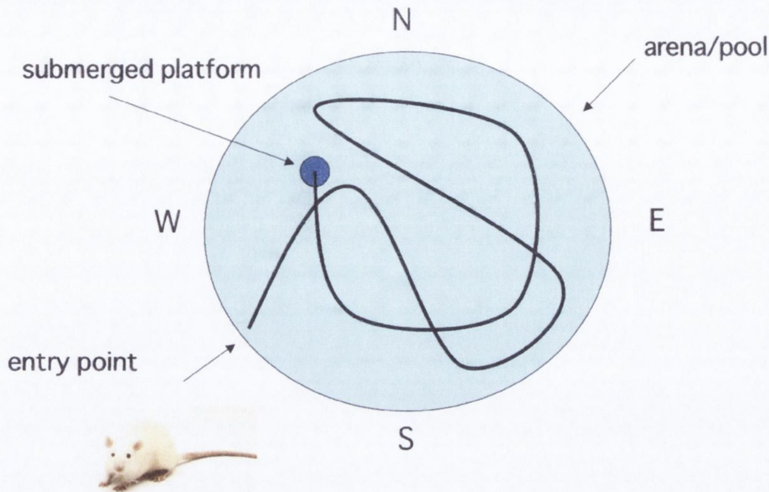


Figure 2.3 Schematic of the MWM

This schematic shows the set up for the MWM. The circular pool had a submerged platform in the NW quadrant.

The MWM was used to assess spatial learning. The water maze was a black circular fibreglass pool (diameter 200cm, height 35cm) filled with water that was at room temperature. The pool was positioned in a room with various extramaze visual cues. The maze was divided into four quadrants; North-East (NE), North-West (NW), South-East (SE) and South-West (SW). The escape platform (a concrete cylinder 30cm in height) was positioned in the NW quadrant and was submerged 2cm below the surface of the water. The hidden platform was always located in the NW quadrant to ensure extramaze visual cues were similar for all trials; this is standard protocol. A schematic of the MWM is shown in Figure 2.3. For the acute exercise studies water maze training began on day 3 of the exercise program and was continued for 5 days consecutively (Figure 2.4). Animals exercised in the morning and performed the MWM task in the afternoon after resting for a minimum of 2hr.

DAY	1	2	3	4	5	6	7
EXERCISE	×	×	×	×	×	×	×
MWM			×	×	×	×	×

Figure 2.4 Timeline of the acute exercise program and the MWM

A 7-day timeline of the acute exercise program and MWM training is shown. From day 3 onwards exercise sessions and MWM training were completed on the same day.

For the chronic exercise studies MWM training was completed in the week following cessation of the exercise program.

MWM training was completed over 5 consecutive days. Each rat was transferred in isolation to the MWM lab where they performed 5 trials per day with an inter-trial interval of 15secs. The animals were placed into the water maze at random entry points facing the maze wall and allowed 60secs to locate the escape platform. If unable to locate the platform within 60secs, the rat was guided to it and left there for 15secs before being removed from the pool. Immediately following the task animals were housed in separate cages until dry and then returned to their normal housing.

Data was gathered using the image analysing software program EthoVision (Noldus Information Technology, Wageningen, Netherlands). This system enables detailed tracking of the animals as they complete the task. Recorded parameters included time taken to find the escape platform (secs), swim speed (cm/secs), pathlength (cm) and time spent in the NW quadrant (secs). Performance for each animal was evaluated by calculating the mean escape latency per day for the 5 trials.

2.7.1 Probe test

On day five of the task all rats also performed a probe test. The submerged

platform was removed from the maze and the animals were placed into the pool for one 60secs probe test. The time spent swimming in the NW quadrant where the escape platform had been located was recorded. For the acute exercise studies, animals exercised early morning on day 5 of the task, completed the MWM task after a rest period of 2hr, and completed the probe test late in the afternoon. For the chronic exercise studies, animals performed the probe test at least 2hr after the routine MWM training on day 5. This design is similar to that used by van Praag and colleagues (1999b, 2005) where the probe test was completed on the last day of training at least 4hr after the last MWM trial.

2.8 Tissue preparation

Animals were killed by decapitation at the end of the LTP experiments and by stunning and decapitation immediately after the behavioural experiments were completed. Once the brain had been removed, the whole hippocampus, dentate gyrus and entorhinal cortex were dissected on ice. For LTP experiments, the hippocampus and dentate gyrus from the ipsilateral and contralateral brain (i.e. tetanized and untetanized respectively) were dissected; analysis was completed in untetanized tissue only. For chronic exercise studies, a small piece of dentate gyrus was dissected and stored in 150-200μl of RNA later solution, which preserves tissue RNA, for analysis of mRNA. Some of the tissue was sliced bidirectionally to a thickness of 350μm using a McIlwain tissue chopper (Campden Instruments Ltd, UK), rinsed three times in Krebs solution (NaCl, 136mM; KCl, 2.54mM; KH_2PO_4, 1.18mM; $Mg_2SO_4.7H_2O$, 1.18mM; $NaHCO_3$, 16mM; Glucose, 10mM; $CaCl_2$, 2mM) and once in Krebs solution containing dimethylsulphoxide (DMSO; 10% vol/vol)) before being stored at -80°C in Krebs DMSO solution for later analysis. Some tissue samples were homogenised (x 10 strokes) in Krebs solution using a 1ml glass homogeniser immediately following dissection. The volume of Krebs solution used for homogenising was 350μl or 400μl for the dentate gyrus, and 650μl for the hippocampus and entorhinal cortex. For chronic exercise studies the weight of the brain, hippocampus, heart and liver were recorded. Organs were weighed immediately following sacrifice of the animal.

2.9 Protein quantification

The protein concentration of tissue samples was assessed using the Bradford method (1976). Samples, analysed in duplicate or triplicate, were mixed with 155μl deionised water in a 96-well plate (microtest plate; Starstedt, Ireland). The standard curve, prepared from a stock solution of 200μg/ml bovine serum albumin (BSA) diluted in deionised water and ranging from 3.125μg/ml to 200μg/ml, along with a blank (deionised water) were loaded onto the same plate. Once samples and standards had been loaded, a Bio-Rad reagent (40μl per well) was added and absorbance was read at 630nm using a 96-well plate reader (EIA Multiwell Reader, Sigma). Regression analysis was used to calculate protein concentrations and values were finally expressed as mg protein/ml. Samples were equalised with Krebs solution to ensure that each sample had a similar protein concentration.

2.10 Enzyme-linked immunosorbent assay (ELISA)

Slices of tissue were thawed on ice, washed in Krebs solution and homogenized in 350-400μl of the same solution, unless previously homogenised. Samples were spun at 14000rpm for 5min and the supernatant was removed.

2.10.1 Analysis of BDNF concentration

For the acute exercise studies BDNF concentration in the supernatant was quantified using the Emax ImmunoAssay system (Promega, Madison, WI). This system detects a minimum of 15.6 pg/ml of BDNF. Briefly, 96-well plates (MaxiSorp; NUNC) were coated overnight at 4°C with 1μg/ml monoclonal BDNF antibody diluted in carbonate coating buffer (pH 9.7). The plates were then blocked with 1x block and sample buffer for 1hr. The plates were subsequently incubated for 2hr with samples and standards, washed and incubated for another 2hr with a polyclonal BDNF antibody (0.5μg/ml). The plates were washed thoroughly and incubated with an anti-IgY horseradish peroxidase-conjugated antibody (1:200) for 1hr. Each reaction was developed using TMB One solution for at least 15min. The reaction was stopped with 1 N HCl and the sample absorbance was measured at 450nm with an ELISA plate reader.

For the chronic exercise studies BDNF concentration in the supernatant was

quantified using the human BDNF Duoset ELISA Development system (R&D Systems, Inc. Minneapolis, USA). This system detects a minimum of 23.4pg/ml of BDNF. 96-well plates (MaxiSorp; NUNC) were coated overnight at 4°C with 2.0μg/ml mouse anti-human BDNF capture antibody diluted in PBS (137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5 nM KH_2PO_4. pH 7.2-7.4, 0.2μm filtered). Plates were blocked with reagent diluent (1% BSA in PBS, pH 7.2-7.4, 0.2μm filtered) for 1hr. Plates were subsequently incubated for 2hr with samples and standards, washed and incubated for another 2hr with a biotinylated mouse anti-human BDNF detection antibody (25ng/ml) diluted in reagent diluent. The plates were washed thoroughly and incubated with Streptavidin HRP (1:200) for 1hr. Each reaction was developed using Substrate Solution (1:1 mixture of colour reagent A and colour reagent B) for at least 15min. The reaction was stopped with 2N H_2SO_4 and the optical density was measured at 450nm with an ELISA plate reader.

A more suitable ELISA kit became available during the course of these studies and is why different systems were used for acute and chronic studies.

2.10.2 Analysis of IL-1β concentration

IL-1β concentration in the supernatant was quantified using the rat IL-1β Duoset ELISA Development system (R&D Systems, Inc. Minneapolis, USA). This system detects a minimum of 3.91 pg/ml of IL-1β. 96-well plates (MaxiSorp; NUNC) were coated overnight at 4°C with 0.8μg/ml goat anti-rat IL-1β capture antibody diluted in PBS (137mM NaCl, 2.7mM KCl, 8.1mMNa_2HPO_4, 1.5mM KH_2PO_4. pH 7.2-7.4, 0.2μm filtered). Plates were blocked with reagent diluent (1% BSA in PBS, pH 7.2-7.4, 0.2μm filtered) for 1hr at room temperature (RT). Plates were subsequently incubated for 2hr with samples and standards, washed and incubated for another 2hr with a biotinylated goat anti-rat IL-1β detection antibody (350ng/ml) diluted in reagent diluent. The plates were washed thoroughly and incubated with Streptavidin HRP (1:200) for 1hr. Each reaction was developed using Substrate Solution (1:1 mixture of colour reagent A and colour reagent B) for at least 15min. The reaction was stopped with 2N H_2SO_4 and the optical density was measured at 450nm with an ELISA plate reader.

2.11 Tissue preparation for gel electrophoresis

Tissue not previously homogenized was thawed quickly, washed 3 times in Krebs solution and homogenized as described earlier. Samples of homogenate were equalized for protein concentration (Bradford, 1976) and 50μl of homogenate was diluted 1:1 with sample buffer (Tris-HCL pH 6.8, 0.5M; SDS, 10%w/v; glycerol, 10% v/v; 2-β-mercaptoethanol, 5% v/v; bromophenol blue, 0.05%w/v). Finally, the samples were boiled for 2-5min.

2.12 Gel electrophoresis

7.5%, 10% or 12% acrylamide gels (see appendix) were set between 2 glass plates and positioned securely in the electrophoresis unit (BioRad Mini-PROTEAN 3, BioRad Laboratories, Hertfordshire, England). The inner and outer reservoirs of the unit were filled with electrode running buffer (composition: Tris base, 25mM; glycine, 200mM; SDS 17mM). The gels were loaded with 10μl prepared sample and 5μl molecular weight marker (Biorad) and run at 30mA per plate for approximately 40min.

2.13 Western Immunoblotting

The gel was removed from the electrophoresis unit and washed gently in transfer buffer (composition: Tris base, 25mM; glycine, 200mM; methanol, 20%v/v; SDS pH 8.3, 0.5%w/v). Nitrocellulose paper (Amersham) and filter paper (Whatman No.3) were cut to fit the size of the gel; 1 piece of nitrocellulose paper and 2 pieces of filter paper were required. The nitrocellulose paper was pre-soaked in deionised water for 5min and then soaked in transfer buffer for a further 5 min, the filter paper was soaked in transfer buffer for 5 min. The gel was carefully positioned between the nitrocellulose paper and filter paper and the second piece of filter paper was layered over the nitrocellulose paper so both outermost layers were filter paper. This "sandwich" was placed on the anode of a semi-dry blotter (Apollo Instruments, Alpha technologies, Dublin, Ireland) previously moistened with transfer buffer and air bubbles were removed. The top of the apparatus, also pre-moistened with transfer buffer, contained the cathode and was firmly closed over the anode. Transfer took 80min and a current of 225mA was used. Once the transfer was complete, blots were blocked for non-specific binding overnight at

4°C with a solution of TBS-T (10ml) containing BSA (5%) and then probed with an antibody raised against a particular protein. Following an incubation period, this was washed off using TBS-T and a secondary HRP-conjugated antibody added. Following a second incubation period, immunoreactive bands were detected using SuperSignal West Dura chemiluminescence reagents (Pierce). After 5min the membranes were exposed to photographic film (Hyperfilm, Amersham, UK) in darkness and developed using a Fuji Processor.

2.13.1 Analysis of Pro-neurotrophin Expression

Expression of the pro-neurotrophin proBDNF was analysed by western immunoblotting. 12% acrylamide gels were used to separate protein bands for proBDNF analysis. Non-specific binding was blocked by incubating the nitrocellulose membrane in 5% BSA at RT with gentle agitation for 2hr. The membrane was incubated at 4°C overnight with the primary antibody mouse recombinant proBDNF IgG (1:500 dilution in TBS-T containing 2% BSA; Alamone). Following 3 x 10min washes in TBS-T, the membrane was incubated for 1hr at RT with the secondary antibody goat anti-rabbit IgG-HRP (1:1000 dilution TBS-T containing 2% BSA; Sigma). After incubation with the secondary antibody the membrane was washed in TBS-T (3 x 10min washes) and exposed to SuperSignal for 5min. Finally, the membrane was exposed to photographic film in a darkroom and the film developed.

2.13.2 Analysis of Receptor Expression

TrkB neurotrophin receptor expression was analysed using a similar protocol to that described above. 7.5% acrylamide gels were cast, loaded with samples, run in the electrophoresis unit and transferred to a nitrocellulose membrane. Immediately following transfer the membrane was washed with deionised water and then TBS-T and blocked in 3% PBS-MLK for 90min at RT with constant agitation. The membrane was incubated with the primary antibody anti-TrkB rabbit polyclonal IgG overnight at 4°C with constant agitation (1:1000 dilution in PBS containing 3% MLK; Upstate). Following incubation the membrane was washed with deionised water and incubated with the secondary antibody goat anti-rabbit IgG-HRP for 90min at RT (1:5000 dilution in 3% PBS-MLK; Sigma).

After incubation with the secondary antibody the membrane was washed first with deionised water, then PBS-T (3 x 5min washes) and finally deionised water before exposure to SuperSignal for 5min. Finally, the membrane was exposed to photographic film in a darkroom and the film developed.

p75 neurotrophin receptor expression was also analysed. 10% acrylamide gels were used to separate protein bands for p75. Non-specific binding was blocked by incubating the nitrocellulose membrane in 5% BSA at RT with gentle agitation for 2hr. The membrane was incubated at 4°C overnight with the primary antibody rabbit polyclonal IgG raised against p75 (1:200 dilution in TBS-T containing 2% BSA). Following 3 x 10min washes in TBS-T, the membrane was incubated for 1hr at RT with the secondary antibody goat anti-rabbit IgG-HRP (1:500 dilution TBS-T containing 2% BSA; Sigma). After incubation with the secondary antibody the membrane was washed in TBS-T (3 x 10min washes) and exposed to SuperSignal for 5min. Finally, the membrane was exposed to photographic film in a darkroom and the film developed. Difficulties arose when attempting to optimise a protocol for the detection of p75 by western immunoblotting, it was difficult to minimise non-specific binding to the antibody and as a result the blots are not always clear.

2.13.3 Analysis of ERK activation

12% acrylamide gels were used to separate protein bands for analysis of phosphorylated ERK (pERK) and total ERK (tERK). There are two isoforms of pERK, p42 and p44. Given that previous studies in this lab, and other published studies have primarily shown changes in the p44 isoform, p44 was analysed here. To analyse for ERK phosphorylation, non-specific binding was blocked overnight at 4°C by incubating the nitrocellulose membrane in TBS-T containing 5% BSA. The membrane was washed 3 times in TBS-T (10min washes) before incubation with the primary antibody (mouse anti-phospho ERK monoclonal IgG; Santa Cruz; 1:3000 in TBS-T containing 2% BSA) for 2hr at RT. After 2hr the membrane was washed again (3 x 10min washes) and then incubated with the secondary antibody (anti-mouse IgG-HRP; Sigma; 1:1000 in TBS-T containing 2% BSA) for 1hr at RT. The membrane was washed as before and incubated with SuperSignal for 5min before exposure to the photographic film and film

development.

Blots were then stripped with an antibody stripping solution (1:10 dilution in dH_2O; Reblot Plus Strong Antibody Stripping Solution; Chemicon) and probed again for tERK expression, this enabled confirmation of equal protein loading. Again, non-specific binding was blocked by incubating the nitrocellulose membrane in 5% BSA at 4°C overnight. The membrane was incubated for 2hr at RT with the primary antibody mouse monoclonal IgG raised against ERK-2 (1:1000 dilution in TBS-T containing 2% BSA; Santa Cruz). Following 3 x 10min washes in TBS-T, the membrane was incubated for 1hr at RT with the secondary antibody anti-mouse IgG-HRP (1:1000 dilution TBS-T containing 2% BSA; Sigma). After incubation with the secondary antibody the membrane was washed in TBS-T (3 x 10min washes) and exposed to SuperSignal for 5min. Finally, the membrane was exposed to photographic film in a darkroom and the film developed.

To calculate ERK activation, p44 and tERK were first normalised to control bands to accommodate for the fact that different exposure time for blots may affect the densitometric measurements. Once data were normalised, p44 was expressed per tERK; values are given as p44/tERK (arbitrary units).

2.13.4 Analysis of β-actin expression

β-actin is a major cytoskeletal protein and the concentration of β-actin within a tissue sample gives an indication of the total protein content within that sample. To normalise protein all data produced by western immunoblotting analysis were expressed per β-actin thereby accommodating for any variations in protein loading between samples.

Blots were stripped with an antibody stripping solution (1:10 dilution in dH_2O; Reblot Plus Strong Antibody Stripping Solution; Chemicon) and probed again for β-actin expression. Non-specific binding was blocked by incubating the nitrocellulose membrane in 5% BSA overnight at 4°C with constant agitation. The membrane was incubated with the primary antibody monoclonal anti-β-actin produced in mouse (1:1000 dilution in TBS-T containing 2% BSA; Sigma). Following 3 x 10min washes in TBS-T, the membrane was incubated for 1hr at RT with the secondary antibody anti-mouse IgG-HRP (1:1000 dilution TBS-T

containing 2% BSA; Sigma). After incubation with the secondary antibody the membrane was washed in TBS-T (3 x 10min washes) and exposed to SuperSignal for 5min. Finally, the membrane was exposed to photographic film in a darkroom and the film developed.

2.14 Densitometric analysis

Densitometric analysis was completed using the ZERO-DScan Image Analysis System (Scanalytics, Fairfax, USA) or the Gel Doc It Imaging System (UVP, Medical Supply Company, Ireland) in combination with Labworks (Lablogics Inc, Mission Viejo, California, USA) to quantify all protein bands.

2.15 Real-time polymerase chain reaction (PCR)

Real-time PCR (RT-PCR) is a technique used to quantify mRNA from tissue sample. This technique detects the accumulation of product during the reaction where it is measured at the exponential phase of the PCR technique.

2.15.1 Total RNA extraction

Isolation of total RNA from rat dentate gyrus samples was completed using Nucleospin RNA II kits (Macherey-Nagel). Dissected tissue was stored in 150-200μl RNA later at 4°C. This served to fix the tissue sample and deactivate any potential RNAses. The dentate gyrus tissue was removed from the RNA later, snap frozen with liquid nitrogen within 28 days, and stored at –80°C. The procedure followed for RNA extraction required all equipment to be cleaned with RNAse Away and rinsed with DEPC water prior to use to prevent contamination. The tissue sample was homogenized in lysis buffer (350μl RA1 buffer and 3.5μl β-mercaptoethanol) using a polytron tissue disrupter (Kinetatica, Switzerland). Next, the lysate was filtered through Nucleospin Filter units and centrifuged for 1min at 11,000g. A volume of 350μl of 70% ethanol was mixed with the lysate using a pipette until dissolution occurred. The total lysate was loaded into a nucleospin II column and centrifuged for 30secs at 11,000g. To desalt the membrane of the column 350μl of membrane desalting buffer was added and the column was centrifuged at 11,000g for 1min. Following this, any potential DNA contamination was digested by applying 95μl DNAse reaction mixture directly

onto the centre of the silica membrane of the column and incubating for 15min at RT. The final steps include a number of washing procedures. 200μl of RA2 buffer (inactivates DNAse) was added and centrifuged for 30secs at 11,000g after which the column was placed into a new collecting tube. A second wash was completed by adding 600μl buffer RA3 to the column and centrifuging for a further 30secs at 11,000g. The final wash was completed by adding 250μl RA3 and centrifuging the column for 2min at 11,000g. The column was then placed in a nuclease-free micro-centrifuge tube, the RNA was eluted with 60μl RNAse-free H_2O and centrifuged for 1min at 11,000g. Extracted RNA was stored at –80°C.

2.15.2 RNA quantification

Following RNA extraction the integrity of the RNA was assessed by electrophoresing a sample of RNA on agarose gels. 1.3g of agarose was added to 130ml of TBE (1.0M Tris, 0.9M Boric acid, 0.01M EDTA) and dissolved by heating the solution in a microwave for 2-3min. On cooling, 1.3μl ethidium bromide was carefully added to the solution and swirled to mix. The solution was poured into a sealed gel tray containing two gel combs and left to set for 30min. After the gel had set the apparatus was flooded with TBE. 3μl of RNA was mixed with 2μl Rnase free H_2O and 1μl of loading dye. 4μl of the diluted RNA was loaded onto a 1% agarose gel and electrophoresed at 90mV for 30min-90min, allowing time for the RNA to separate fully. By passing UV light over the gel 2 ribosomal RNA (rRNA) bands are observed, a dense 28S band and a lighter 18S band, the presence of these bands confirms that RNA is present in the sample and has not been degraded.

The optical density (OD) of RNA was quantified using a spectrophotometer (UV/vis Beckman Coulter Du730). RNA was diluted (1:200 dilution in DEPC H_2O) for analysis. RNA absorbs light at 260nm and an OD of 1.0 at 260nm is equivalent to an RNA concentration of 40μg/ml. Thus, the concentration of RNA in sample tissue can be calculated by RNA = OD_{260} x dilution factor x 40μg/ml. The purity of RNA may also be quantified by measuring RNA absorbance at 280nm and calculating the OD_{260}/OD_{280}. A ratio between 1.8-2.1 (approximately) indicates the RNA is pure. Finally, RNA concentrations were equalised prior to reverse transcription.

2.15.3 cDNA preparation

Following RNA equalization, samples were prepared for cDNA production using the ABI High Capacity cDNA kit (Applied Biosystems). This is a sensitive kit and synthesizes 40μl of cDNA. A master-mix solution containing reverse transcription buffer (1:5 dilution), 25x dNTPs (1:12.5 dilution), random primers (1:5 dilution), multiscribe reverse transcriptase (1:10 dilution) and RNAse free H_20 (1:2.38 dilution) was prepared and stored on ice. 20μl of 0.5-2.5μg RNA was added to 20μl of the master mix in PCR tubes. The samples were placed in a thermocycler and incubated for 10min at 25°C and then 120min at 37°C. This completed the amplification stage and samples were stored at –20°C.

2.15.4 RT-PCR

RT-PCR was performed using Taqman Gene Expression Assays (Applied Biosystems, USA). These contain specific target primers and FAM-labelled MGB target probes. β-actin gene expression was measured to normalise gene expression between samples. For multi-target (multiplex) QPCR cDNA was thawed and diluted with sigma water (1:4 dilution). A reaction volume of 25μl was added to each well of the PCR plate (10μl of diluted cDNA, 1.25μl of primer/probe, 1.25μl of β-actin primer/probe and 12.5μl of Taqman® Universal PCR Master Mix). Electronic pipettes (EDP3 20-200μl, 10-100μl and 2-20μl) were used to ensure pipetting accuracy. All RT-PCR measurements were conducted using an ABI Prism 7300 instrument (Applied Biosystems). Samples were positioned in the PCR thermocycler and 40 cycles were repeated for step 2 of the program. The program was composed of different stages, step 1: 95°C for 10min and step 2: 95°C for 15secs followed by the final transcription step at 60°C for 1min for each cycle (denaturation). Fluorescence was read during the annealing and extension phase (60°C) throughout the program.

Gene	Assay
BDNF	Rn00560868_m1
NT3	Rn00579280_m1
NT4/5	Rn01645105_m1
TrkA	Rn00572130_m1
TrkB	Rn00820626_m1
TrkC	Rn00570389_m1
P75	Rn00561634_m1
IGF-1	Rn00710306_m1
VEGF	Rn00582935_m1
Ki-67	Rn01451446_m1
IL-1β	Rn00580432_m1
IκB	Rn00578384_m1
TNF-α	Rn99999017_m1

Table 2.1 Taqman gene expression assays used

To outline the various stages of the PCR reaction in more detail, initially during step 1 samples are heated to 95°C for 15secs to denature the double stranded cDNA. Following this, the temperature of the reaction decreases to allow annealing and extension of the cDNA. The target probe is the first to anneal to the single-stranded cDNA as it has a higher melting temperature than the target primers. This probe contains a FAM/VIC dye and a proprietary non-fluorescent quencher (NFQ) dye. The quencher blocks the FAM/VIC dye from emitting a fluorescent signal by fluorescence resonance energy transfer (FRET) technology (Applied Biosystems). Once the reaction temperature reaches 60°C the primers anneal to the strand of cDNA and it is extended by 5' nuclease activity of the Taq polymerase. This process induces the release of the FAM/VIC-labelled probe and causes the FRET between the dye and quencher to be broken which subsequently allows the generation of a fluorescent signal. Due to the specificity of the probe and primers for the cDNA sequence, one fluorescent signal is produced for each new cDNA copy and measured during the annealing stage of the PCR cycle

(60°C).

2.15.5 RT-PCR analysis

The ΔΔCT method (Applied Biosystems RQ software, Applied Biosystems, UK) was used to assess gene expression for all RT-PCR analysis. This method assesses relative gene expression by comparing gene expression of treated/experimental samples to an untreated sample (control). Analysis of the fold-difference (increase or decrease) can be compared between experimental and control samples. The fold-difference is assessed using the cycle number (CT) difference between samples. Briefly, a threshold for fluorescence is set and CT is measured against this value. To allow accurate analysis of the differences in gene expression the threshold is set when the PCR reaction is in the exponential phase i.e. when the PCR reaction is optimal or 100% efficient. Effectively, samples with low CT readings demonstrate high fluorescence that indicates greater amplification and greater gene expression. When a PCR is 100% efficient a one-cycle difference between samples means a 2-fold difference in copy number (2^2), similarly a 5-fold difference is a 32-fold difference (2^5).

To measure this fold-difference relative to control, the CT of the endogenous control (β-actin) is subtracted from the CT of the target gene for each sample to account for any difference in cDNA quantity. This normalised CT value is called the (CT). The CT difference (ΔCT) of the control is subtracted from itself to give 0 and subtracted from all other samples, to give the ΔΔCT value. The ΔΔCT (cycle difference corrected for β-actin) is then converted into a fold-difference. As a one-cycle difference corresponds to a two-fold increase or decrease relative to control, 2 to the power of the ΔΔCT gives the fold-difference in gene expression between the control and experimental samples. The control sample always has a ΔΔCT value of 0, thus 0^2 gives a $2^{\Delta\Delta CT}$ of 1, against which all other samples are referenced. Data are presented as relative quotient (RQ) values that represent fold changes relative to the mean value for controls.

	Target CT	β-actin CT	ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$ Fold difference
Control	24	17	24-17 = 7	7-7 = 0	2^{-0} = 1
Sample 1	28	17	28-17 = 11	11-7 = 4	2^{-4} = 0.0625
Sample 2	22	17	22-17 = 5	5-7 = -2	$2^{-(-2)}$ = 4
Sample 3	20	17	20-17 = 3	3-7 = -4	$2^{-(-4)}$ = 16
Sample 4	29	17	29-17 = 12	12-7 = 5	2^{-5} = 0.03125

Table 2.2 An example of the method used for ΔΔCT analysis

2.16 Data analysis

Data were analysed using the software programs Microsoft Excel and GraphPad Prism. When comparing exercise and control groups within a specific population an unpaired Student's *t*-test was used. To analyse the effect of exercise on LTP a 2-way analysis of variance (ANOVA) post tetanus was used. In addition, the average value for % EPSP during the last 5min of the experiment was calculated to give an indication of the ability to sustain LTP; control and exercised groups were compared using an unpaired Student's *t*-test. To determine the effect of exercise on object recognition memory or spatial learning, a 1- or 2-way ANOVA was used where appropriate. To determine the effect of exercise on measured cellular parameters data were analysed using an unpaired Student's *t*-test. When analysing age-related differences a 1-way ANOVA was used. For analysis by ANOVA, post-hoc comparisons were calculated using the Neuman-Keuls test. A value of $p<0.05$ was considered to be significant. All data are presented as mean ± S.E.M.

When analysing the effect of exercise or age on measured cellular parameters data from LTP and behavioural experiments were analysed individually. Where no differences were observed data from LTP and behavioural experiments were pooled from control animals, and separately from exercised animals, to assess whether a larger sample size would increase statistical power and reveal significant differences. While it is appreciated that surgical procedures and learning may influence the level of cellular proteins, data were only pooled when

no differences were observed between LTP and behavioural experiments.

Chapter 3

An investigation into the effect of an acute exercise program on LTP, recognition memory and spatial learning in young animals; a role for BDNF?

Chapter 3

An investigation into the effect of an acute exercise program on LTP, recognition memory and spatial learning in young animals; a role for BDNF?

3.1 Introduction

It has been suggested that exercise has a positive effect on neuronal function and has the potential to improve cognitive function. It appears that regular exercise, in addition to improving fitness and promoting general good health, may improve cognitive function and reduce the risk of dementia in human populations (Rovio *et al.*, 2005; Stevens & Killeen, 2006). Human research in this field has investigated the effects of exercise on aged populations that are both cognitively sound and those showing existing cognitive impairment. Observational studies have produced strong evidence that exercise may ameliorate the cognitive decline associated with dementia and Alzheimer's disease and possibly reduce the risk of developing dementia (Abbott *et al.*, 2004; Heyn *et al.*, 2004).

Animal work has facilitated more detailed analysis of the underlying mechanisms responsible for these effects on the brain. Studies have explored the effects of exercise on hippocampal synaptic plasticity and various forms of hippocampal-dependent memory. An enhancement of LTP has been observed following exercise programs of varying durations (Farmer *et al.*, 2004; van Praag *et al.*, 1999b). Although not in every case, exercise has been shown to positively influence spatial learning assessed by the MWM (Adlard *et al.*, 2004; Vaynman *et al.*, 2004b) and the radial arm maze (Anderson *et al.*, 2000). Another form of hippocampal-dependent learning that is enhanced by exercise is contextual fear conditioning. Baruch and colleagues (2004) demonstrated an improvement in contextual fear conditioning following 30 days of voluntary exercise. Research that has investigated the molecular mechanisms underlying exercise-induced cognitive changes has suggested that the neurotrophin BDNF is a central mediator of the exercise-related effects on cognitive function. Several studies have reported that BDNF plays a key role in mediating the exercise-induced effects on brain plasticity (Berchtold *et al.*, 2005; Molteni *et al.*, 2002; Vaynman *et al.*, 2003). Exercise-induced BDNF signalling has also been explored and a multitude of signalling pathways, signalling molecules and transcription factors have been

implicated. Shen and colleagues (2001) observed an increase in phosphorylated MAPK and the level of activated CREB following a variety of exercising periods. Interestingly, the time-course of increase for these two molecules was different. The PI-3K pathway has also been associated with the exercise-induced increase in BDNF (Chen *et al.*, 2005).

The purpose of this study was to determine the effects of an acute 7-day forced exercise program on LTP, object recognition memory and spatial learning in a young rodent population. The cellular mechanisms that underlie the exercise-induced effects on cognitive function were investigated. More specifically, changes in the expression of BDNF, proBDNF, neurotrophin receptor sub-types and associated signalling molecules were explored.

3.2 Materials and Methods

4-month-old male Wistar rats, an inbred strain supplied by the Bioresources Unit (BRU) of Trinity College Dublin, weighing between 250-350g, were used in these studies. Animals were acclimatised to their new environment before commencing the exercise program.

Following familiarisation to the motorised treadmills (Exer 3/6 treadmill, Columbus Instruments, USA) animals were equally divided into control and exercising groups. The acute exercise program consisted of 7 days consecutive running at a speed of 17m/min for 60min (approximately 1km per day). Control rats were placed on stationary treadmills for the same duration. Following the exercise program, animals were either assessed for LTP (n=12; 6 control, 6 exercised), recognition memory (n=12; 6 control, 6 exercised) or spatial learning (n=12; 6 control, 6 exercised).

The LTP experiments were staggered to ensure that all assessments were completed the day after the exercise program was finished. Assessment of recognition memory was also completed the day after the exercise program was completed. The MWM task, assessing spatial learning, was commenced on day 3 of the exercise program and continued for 5 days consecutively. Details of LTP experiments, the object recognition task and the MWM task are given in sections 2.5, 2.6 and 2.7 respectively.

Following sacrifice of the animals, dentate gyrus, whole hippocampus and

entorhinal cortex were dissected on ice and stored at -80°C for further analysis (section 2.8). Tissue homogenate (dentate gyrus, hippocampus and entorhinal cortex) from LTP, object recognition and MWM experiments was thawed and spun at 14,000 rpm for 5min to extract the supernatant for analysis of BDNF concentration by ELISA (section 2.10 and 2.10.1). Dentate gyrus samples from LTP and MWM experiments were analysed for the expression of proBDNF, the neurotrophin receptor subtypes (TrkB and p75) and ERK by gel electrophoresis and western immunoblotting (sections 2.11, 2.12, 2.13 and 2.14).

When identifying cellular changes experiments were analysed individually. Where no effect was observed data from LTP and behavioural experiments were pooled from control animals, and separately from exercised animals, to identify if an exercise effect would be revealed with a larger sample size. Pooled data are presented unless otherwise specified.

Results 3.3

3.3.1 Effect of exercise on LTP

The effect of an acute 7-day forced exercise program on LTP in the dentate gyrus of young rats was assessed. No significant exercise-effect was observed when data was analysed using a 2-way ANOVA (Figure 3.1a). However, using a Student's *t*-test to compare average values for % EPSP during the last 5min of the experiment, giving an indication of the ability to sustain LTP, revealed a significant exercise-induced effect (Figure 3.1b). The mean % EPSP (± SEM) during the last 5min of the experiment showed a significant exercise-induced enhancement in LTP when comparing control (115.1 ± 2.49 %) and exercised (132.8 ± 6.40 %) groups. The experiments completed show a smaller magnitude of LTP in young control animals than was expected. In a similar study, Farmer and colleagues (2004) reported a 44.8 ± 15 % change in EPSP immediately following a conditioning stimulus in young control rodents.

3.3.1.1 Effect of exercise on object recognition memory

The effect of a 7-day forced exercise program on object recognition memory was assessed by a 2-day object exploration task. On day 1 of the task both groups explored object A and B for similar % time (Figure 3.2a). On day 2 (Figure 3.2b)

both groups explored the novel object (C) for significantly longer % time than that spent exploring object A ($p<0.001$; 36.8 vs 63.2 % and $p<0.001$; 24.0 vs 76.0 %, for control and exercise groups respectively). The exercise group showed significantly greater preference for object C than age-matched controls ($p<0.001$). This result indicates that more efficient learning had taken place in the exercising animals. On day 2 of the task, the total exploration time was 23.8 ± 8.4 and 28.8 ± 6.2 secs for control and exercising animals respectively; there was no significant difference between groups.

3.3.1.2 Effect of exercise on spatial learning

The MWM was used to assess the effect of acute exercise on spatial learning. Figure 3.3a shows the performance, given by escape latency, of control and exercised rats during the 5-day task. Data from control and exercised groups were analysed by 1-way ANOVA to identify the effect of day on escape latency. It is clear that all animals learnt the task as mean escape latency was significantly reduced ($p<0.01$) on day 1 (49 ± 5 and 47 ± 3 secs for control and exercised groups respectively) compared with day 5 (23 ± 7 and 21 ± 2 secs for control and exercised groups respectively). To identify the effect of day and exercise on escape latency data were analysed by 2-way ANOVA. The results show no effect of exercise on MWM performance. Data for the probe test revealed that both groups had a preference for the NW quadrant compared to other quadrants in the pool (Figure 3.3b & c). This is most evident for exercising animals (Figure 3.3c). Exercising animals swam in the NW quadrant for significantly more percentage time than all other quadrants ($p<0.001$ for NW compared to SW and NE and $p<0.01$ for NW compared to SE). Results for the time spent swimming in the NW quadrant did not differ significantly between groups (Figure 3.3d; 21 ± 4 and 26 ± 3 secs for control and exercise groups respectively). This result is consistent with escape latency data and suggests that exercise did not affect this type of learning.
Swim speed (cm/secs) and pathlength (cm) were also recorded. These data were analysed by 2-way ANOVA to determine the effect of day and exercise on swim speed or day and exercise on pathlength. There were no differences observed between control and exercised animals for pathlength over the 5-day task. As expected, swim speed was increased for both groups over the 5-day task. On day

4 controls animals were significantly faster ($p<0.05$) than exercised animals (17 ± 1 and 20 ± 1 secs for control and exercised animals respectively).

3.3.2 Effect of exercise on BDNF concentration

The impact of short-term exercise on the concentration of BDNF was investigated. The exercise-induced improvement in hippocampal function is associated with an increase in BDNF concentration in the dentate gyrus (Figure 3.4a). A significant difference between control and exercising groups was observed ($p<0.05$). The concentration of BDNF in the dentate gyrus was 0.18 ± 0.03 and 0.28 ± 0.04 ng/mg for control and exercised animals respectively.

Specific to the LTP experiments there was an increase in BDNF concentration in the hippocampus ($p<0.01$). BDNF concentration was 0.14 ± 0.02 and 0.26 ± 0.02 ng/mg for control and exercising groups respectively (Figure 3.4b). Interestingly, there was also an exercise-induced increase in the concentration of BDNF in the hippocampus and entorhinal cortex for object recognition experiments (Figure 3.4c, d & e). Following the 2-day object recognition task BDNF concentration was 0.18 ± 0.05 and 0.43 ± 0.04 ng/mg in the dentate gyrus of control and exercise groups respectively (Figure 3.4c). BDNF concentration was 0.16 ± 0.04 and 0.32 ± 0.01 ng/mg in the hippocampus of control and exercised groups respectively (Figure 3.4d). In the entorhinal cortex (Figure 3.4e) BDNF concentration was 0.14 ± 0.04 and 0.33 ± 0.03 ng/mg for exercising and control animals respectively.

3.3.3 Effect of exercise on proBDNF expression

The effect of an acute forced exercise program on the expression of proBDNF in the dentate gyrus was assessed. Although no significant differences were observed between control and exercising animals, it appears that there may be a trend for an increase in proBDNF expression in response to acute exercise. In the dentate gyrus, the expression of proBDNF for control animals was 0.82 ± 0.12 proBDNF/β-actin, and for exercising animals was 1.48 ± 0.46 proBDNF/β-actin (Figure 3.5).

3.3.4 Effect of exercise on neurotrophin receptor sub-types

The effect of acute forced exercise on the expression of TrkB and the p75 receptor in the dentate gyrus was assessed. There was no effect of acute exercise on either of the neurotrophin receptor sub-types. The expression of TrkB (Figure 3.6a) was 0.54 ± 0.16 TrkB/β-actin for control animals, and 0.42 ± 0.09 TrkB/β-actin for exercising animals. The expression of p75 (Figure 3.6b) was 0.16 ± 0.05 p75/β-actin for control animals, and 0.12 ± 0.04 p75/β-actin for exercised animals.

3.3.5 Effect of exercise on ERK activation

The effect of acute exercise on the activation of ERK within the dentate gyrus was assessed. There was no change in ERK activation following acute exercise (Figure 3.7). ERK activation was 0.23 ± 0.02 and 0.23 ± 0.03 p44/tERK (arbitrary units) for control and exercising rats respectively.

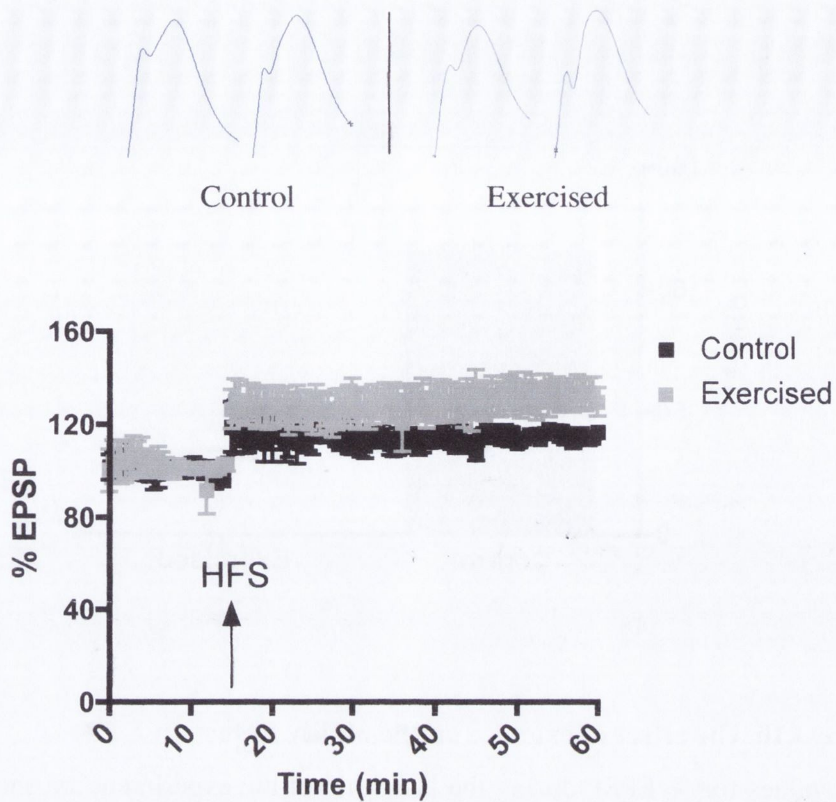


Figure 3.1a The effect of exercise on LTP

Figure 3.1a shows mean data (± SEM) for each test shock delivered pre and post HFS. Results are expressed as % EPSP slope normalised to baseline recordings. LTP was sustained for the duration of the experiment in both control (n=6) and exercised (n=6) groups. No exercise-induced effect was observed when data were analysed using a 2-way ANOVA analysis post tetanus.

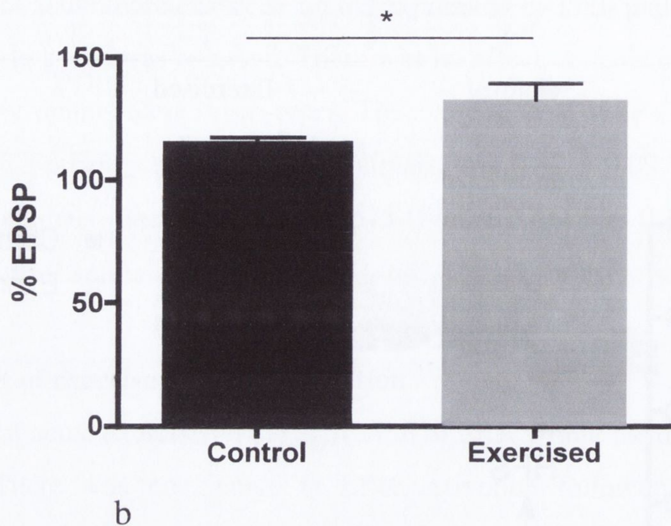


Figure 3.1b The effect of exercise on the ability to sustain LTP

Mean values for % EPSP during the last 5min of the experiment are shown. This data gives an indication of the ability to sustain LTP. Mean values (± SEM) are presented; an unpaired Student's *t*-test was used for statistical analysis. The mean % EPSP during the last 5min of the experiment was significantly greater ($p<0.05$) in the exercise group (n=6) when compared with controls (n=6) suggesting that exercise produces an enhancement in LTP.

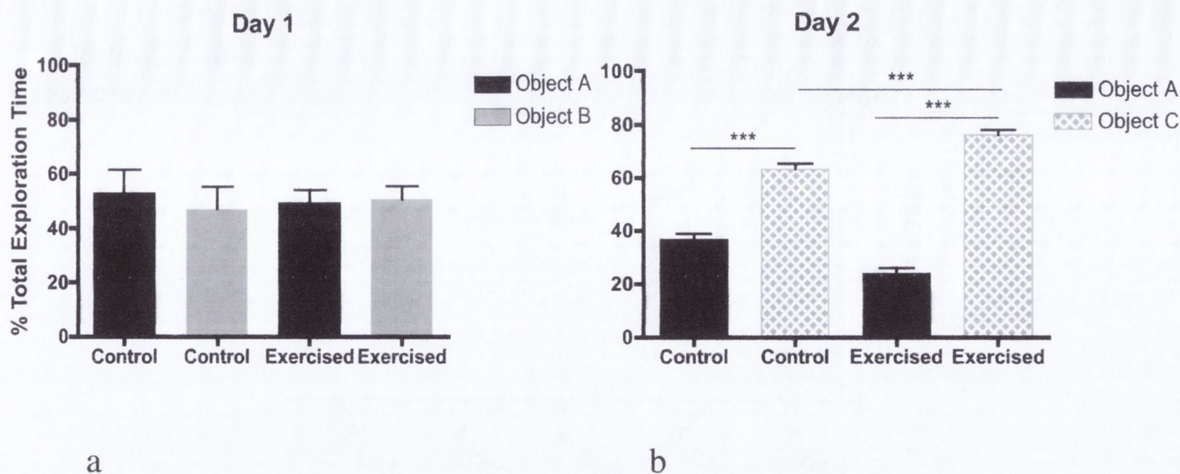


Figure 3.2a & b The effect of exercise on object recognition memory

The % of total time spent exploring objects A and B on day 1, and objects A and C on day 2, for both control (n=6) and exercised (n=6) groups are shown. Results are expressed as mean values ± (SEM), for statistical analysis a 1-way ANOVA and post hoc Neuman-Keuls test was used. On day 1 exercise did not affect the exploration of objects. On day 2 both groups demonstrated a significant difference ($p<0.001$) in the % time spent exploring the novel object (C). In addition, exercised rats explored the novel object more than the control group ($p<0.001$) suggesting that an exercise-induced enhancement in learning had occurred.

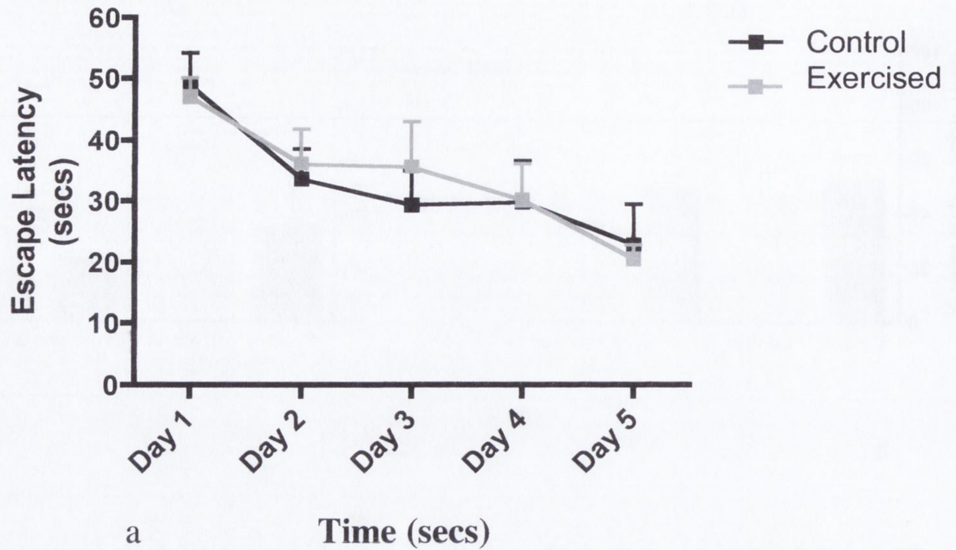


Figure 3.3a The effect of exercise on MWM performance

MWM performance for both exercised (n=6) and control (n=6) groups is shown. Results are given as mean values (± SEM). For statistical analysis, a 1-way ANOVA was used to determine the effect of day on escape latency for separate groups, and a 2-way ANOVA was used to determine the effect of day and exercise on escape latency; post hoc analysis was completed using the Neuman-Keuls test. Both groups show a significant decrease in escape latency over the 5-day task suggesting that learning has occurred. No exercise-related improvement in spatial learning was observed.

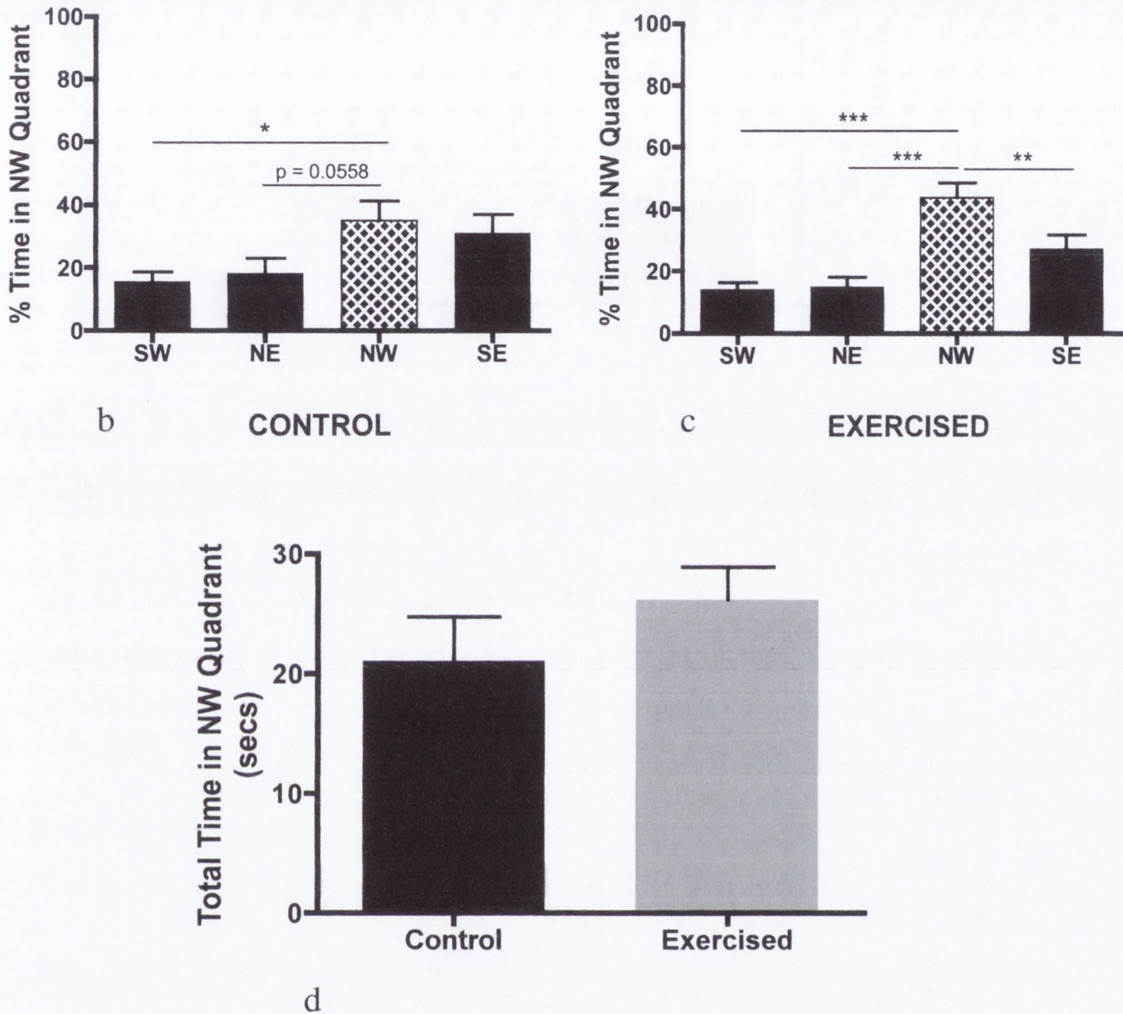
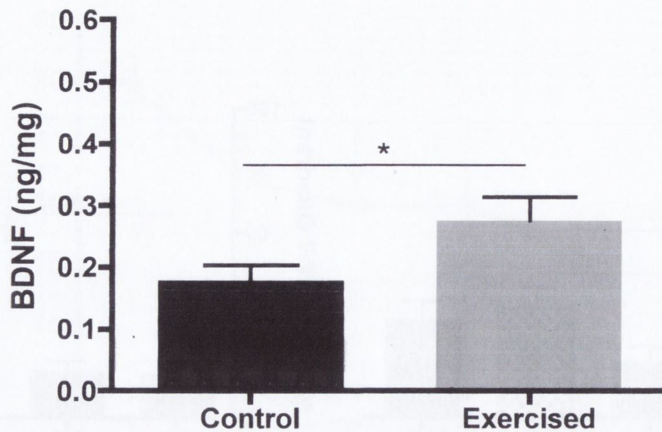


Figure 3.3b, c & d Performance in the probe test; a comparison of exercised and control groups

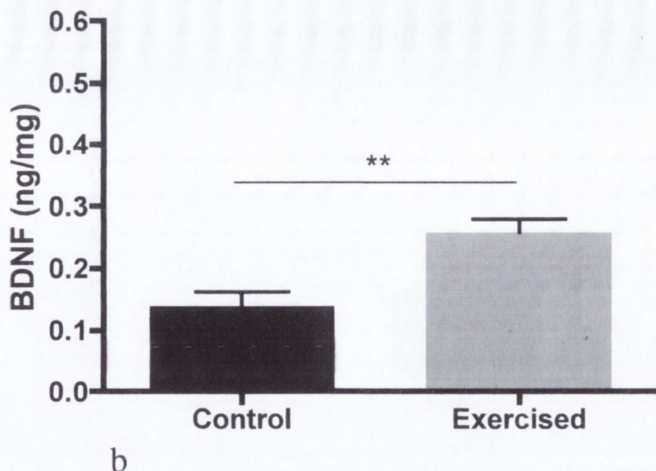
Results for % of total time swimming in each pool quadrant for the 60 secs probe test are presented along with the total time spent swimming in the NW quadrant; data for control (n=6) and exercising (n=6) animals are shown. Results are expressed as mean values (± SEM), statistical analysis was completed using a 1-way ANOVA or an unpaired Student's *t*-test where appropriate. Both groups show a significant preference for the NW quadrant, this is more evident with exercised animals. However, there was no difference in the total time swimming in the NW quadrant, which would suggest similar learning had occurred.



a

Figure 3.4a The effect of exercise on the concentration of BDNF in the dentate gyrus

The results for the concentration of BDNF in the dentate gyrus of control (n=18) and exercised (n=18) animals are shown. Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was a significant increase in BDNF concentration following acute exercise ($p<0.05$).



b

Figure 3.4b The effect of exercise on the concentration of BDNF in the hippocampus; LTP experiments

The results for the concentration of BDNF in the hippocampus of both control (n=6) and exercise (n=6) groups for LTP experiments are shown. Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. BDNF concentration was significantly greater in exercising animals when compared with controls ($p<0.01$).

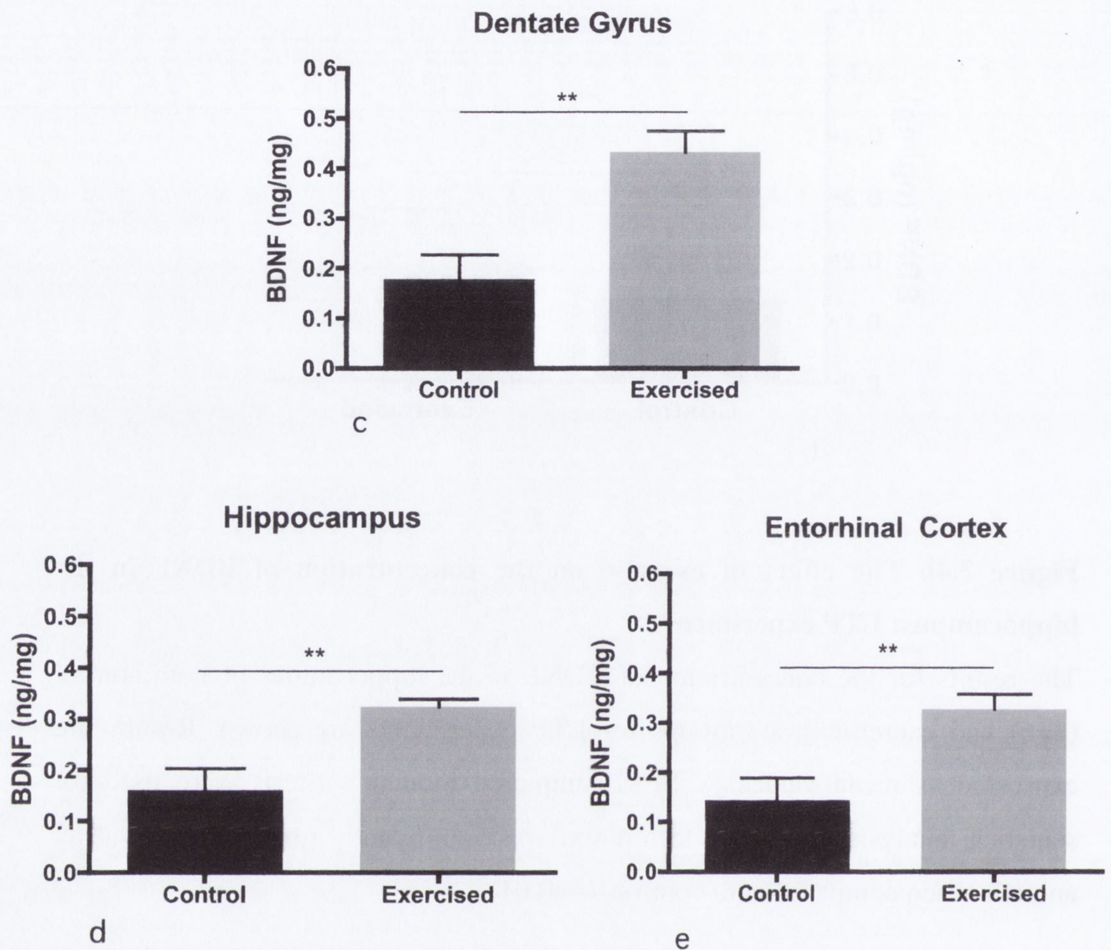


Figure 3.4c, d & e The concentration of BDNF in the dentate gyrus, hippocampus and entorhinal cortex; 2-day object recognition task

The results for the concentration of BDNF in the hippocampus, dentate gyrus and entorhinal cortex from both control (n=6) and exercising (n=6) groups following the 2-day object recognition task are shown. Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. Within the dentate gyrus a significant increase ($p<0.01$) in BDNF concentration was observed in the exercise group compared with the control group. Similarly, in the hippocampus BDNF concentration was significantly increased ($p<0.01$) in the exercise group compared with controls. There was also an exercise-induced increase in BDNF concentration in the entorhinal cortex ($p<0.01$).

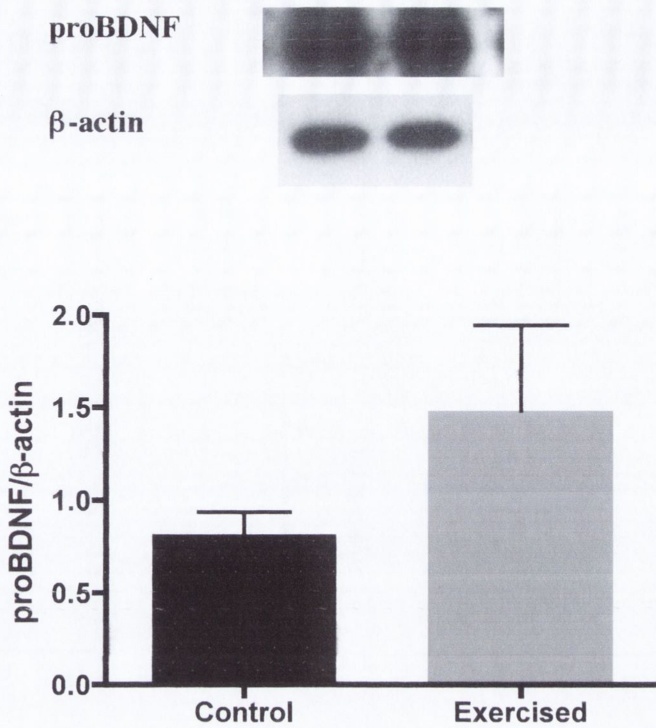


Figure 3.5 The effect of exercise on the expression of proBDNF

The results for the expression of proBDNF in the dentate gyrus of both control (n=12) and exercise (n=12) groups are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. No exercise-effect was observed.

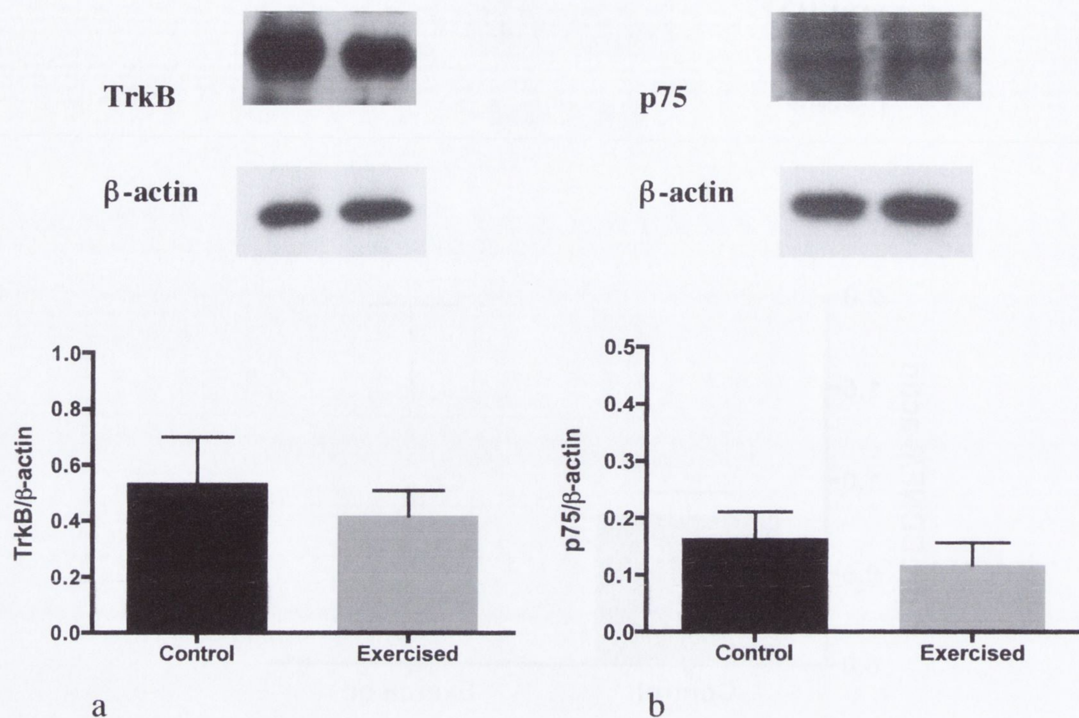


Figure 3.6a & 3.6b The effect of exercise on the expression of TrkB and p75
The results for the expression of TrkB and p75 neurotrophin receptor sub-types in the dentate gyrus of both control (n=12) and exercise (n=12) groups are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was no effect of acute exercise on the expression of either neurotrophin receptor sub-type.

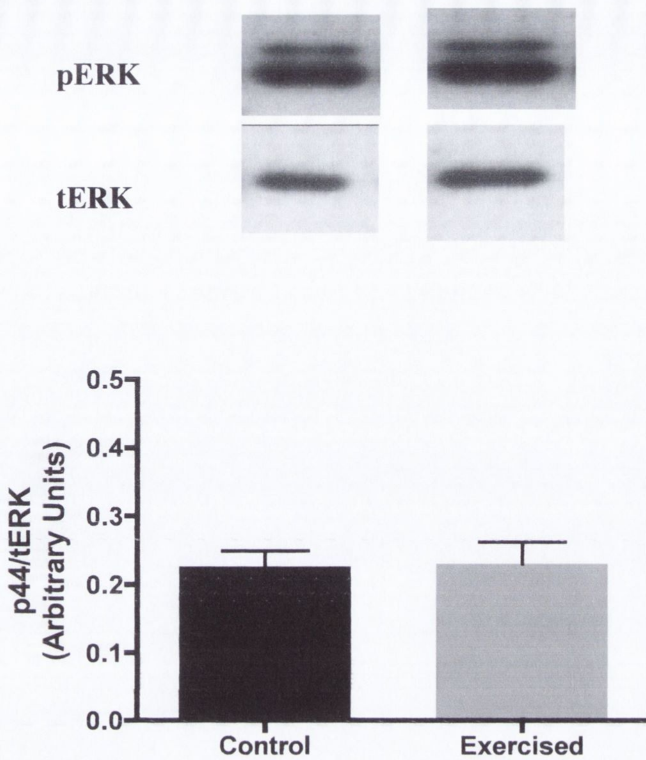


Figure 3.7 The effect of exercise on ERK activation

Results for ERK activation in control (n=12) and exercised (n=12) groups following acute exercise are shown. Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was no exercise-induced change in ERK activation. The protein bands for control and exercising rodents were taken from the same blot. However, when gels were loaded an attempt was made to randomise samples and as a result consecutive wells did not always contain control and exercising samples.

3.4 Discussion

The aim of these experiments was to determine the effect of an acute 7-day forced exercise regime on synaptic plasticity and different forms of learning in a young rodent population. Biochemical analysis was completed to better understand how exercise mediates its effects on cognitive function. Specifically, investigations were focused on the role of BDNF and associated signalling pathways.

The results demonstrate an improvement in some forms of hippocampal function following a short-term forced exercise program in young animals. LTP and recognition memory were enhanced in response to acute exercise. The exercise-induced effect on hippocampal function was associated with an increase in BDNF concentration in the dentate gyrus. There were no significant changes in the expression of proBDNF, neurotrophin receptor sub-types TrkB and p75, or ERK activation following exercise.

3.4.1 The effect of acute exercise on hippocampal function

The significant enhancement in LTP following exercise observed in this study is consistent with other literature. Both van Praag and colleagues (1999b) and Farmer and colleagues (2004) found that physical activity enhances LTP in young adult rodents. Van Praag and colleagues (1999b) demonstrated a significant exercise-induced enhancement in EPSP amplitude (% change) in the dentate gyrus. Interestingly, no differences were found between control and exercising groups in the initial EPSPs that would suggest there was no effect of running on basal synaptic efficiency. However, at 45min post HFS there was a significant difference between the groups. Farmer and colleagues (2004) showed a significant exercise-induced effect on LTP. The change in % EPSP was 56.2 ± 9.7 % for runners. This study also revealed that weak theta-patterned stimulation unable to stimulate LTP in control animals, produced a robust long-lasting LTP in exercised animals. On the basis of these results, the authors suggest that LTP is easier to obtain in animals that have engaged in voluntary exercise by way of an alteration in the induction threshold for synaptic activity.

A particularly promising result produced by these experiments was an exercise-induced increase in recognition memory. There is no other published research that specifically explores the effect of exercise on this form of learning in young

healthy animals and this data further strengthens the existing hypothesis that exercise positively affects hippocampal function. Recognition memory was assessed using a 2-day object recognition task. Animals in the exercise group spent significantly more time exploring the novel object (C) on day 2 of the task when compared with age-matched controls, which suggests an exercise-related improvement in learning. This effect was not attributed to a greater total exploration time by the exercising animals; total exploration time was similar for both groups.

A number of previous studies have explored the effects of exercise on spatial learning. The experiments completed here show no evidence of an exercise effect on spatial learning, which is somewhat in contrast with other literature. These data indicate that exercised animals had a more obvious preference for the NW quadrant during the probe test, measuring retention memory. However, a comparison of total time in the NW quadrant (a standard assessment of performance in the probe test) was not different between groups.

A number of studies have demonstrated improvements in spatial learning in response to exercise (Anderson *et al.*, 2000; Fordyce & Farrer, 1991; Vaynman *et al.*, 2004b). It is important to consider that a wide diversity in exercise regimes exists and may account for the differences observed in these experiments. Exercise mode, duration, intensity and frequency are highly variable. Kim and colleagues (2003) demonstrate that cell proliferation in the dentate gyrus of rats is modulated by the intensity and duration of exercise, highlighting that different exercise programs may differentially impact the brain. It appears that many studies showing an exercise-induced improvement in spatial learning have used exercise programs of longer duration than that used in these studies (Anderson *et al.*, 2000; Ang *et al.*, 2006; Fordyce & Farrer, 1991; van Praag *et al.*, 1999b;). Thus, it is possible that an extended exercise period is necessary for improvements in spatial learning to be consistently observed in young rodents.

Another likely explanation for the result that no exercise-effect on spatial learning was observed in the current experiments, is that young control animals with intact spatial learning would presumably perform well in the water maze, and it is possible that the assessment of spatial learning used here was not challenging enough to dissociate an exercise effect. Van Praag and colleagues (1999b) studied

3-month-old mice engaging in 2-4 months of voluntary exercise and observed a significant improvement in spatial learning, assessed by MWM, in the exercising groups. Interestingly, when mice were trained with 4 trials per day no exercise-effect on pathlength was observed. However, using a more challenging 2-trials-per-day paradigm, revealed an exercise-induced improvement in acquisition, demonstrated by a reduced pathlength and escape latency. Perhaps, if the MWM task used in these experiments were similarly modified to produce a more challenging assessment of spatial learning, by reducing the number of trails-per-day, an exercise effect would have been observed.

Another important consideration is that spatial learning may more noticeably be improved by exercise when an impairment in spatial learning exists prior to the exercise intervention. Perhaps the potential for exercise to ameliorate a deficit in spatial learning (either age-related or experimentally induced) and play a "neuroprotective" role more accurately describes the effects of exercise on some forms of hippocampal learning. Exercise-related improvements in learning and memory have been consistently reported in aged animals (Albeck *et al.*, 2006; Radak *et al.*, 2001). Additionally, where spatial learning deficits are induced experimentally in young rats, exercise has been shown to improve spatial learning (Gobbo & O'Mara, 2005). It may be speculated that exercise does not always induce cognitive enhancement, but more consistently offers neuroprotection against neurodegenerative decline. Indeed, Christie and colleagues (2005) suggested that voluntary exercise could have therapeutic benefits by rescuing deficits in both spatial memory and LTP.

3.4.2 How are the exercise-induced effects on hippocampal function mediated?

A variety of stimuli that enhance neuronal activity may increase the concentration and expression of BDNF (Castren *et al.*, 1998; Gwag & Springer, 1993). The exercise-induced improvement in hippocampal function that was observed in these experiments was associated with an increase in the concentration of BDNF in the dentate gyrus; a result that supports the weight of research promoting BDNF as a key mediator of the exercise-induced effects on neuronal function. Many authors have suggested that BDNF plays a crucial role in mediating the

effects of exercise on cognitive function (Farmer *et al.*, 2004; Gomez-Pinella *et al.*, 2002; Vaynman *et al.*, 2004b). In addition, specific to LTP experiments, an exercise-induced increase in BDNF concentration in the hippocampus was demonstrated. For object recognition studies, an exercise-induced increase in BDNF concentration was also observed in the hippocampus and entorhinal cortex. Regardless of the stimulus, it is interesting to speculate that cellular modifications associated with an enhancement in synaptic plasticity, an improvement in recognition learning, or other forms of learning may be specific to regions of the hippocampus. Oliff and colleagues (1998) report that in some instances exercise-induced up-regulation of BDNF is specific to a particular hippocampal region. Other research has shown that exercise-induced changes in BDNF expression in the cerebellum are specific to particular regions of the cerebellum (Klintsova *et al.*, 2004). In addition, Widenfalk and colleagues (1999) produced interesting data showing that where exercise produces an increase in BDNF mRNA in the hippocampus, an abrupt interruption of prolonged spontaneous exercise results in a decrease in the expression of mRNA encoding for BDNF in certain hippocampal areas. The most prominent down-regulation was observed in the medial CA3 region. These results lend support to the contention that exercise-induced cellular modifications are region specific. In addition, it is likely that where cellular changes occur in a particular region of the hippocampus, or cerebellum, that these modifications are associated with a specific functional role.

It is well established that BDNF/TrkB signalling plays a fundamental role in mediating the enduring changes in central synaptic function and structure (Tyler *et al.*, 2002) and is crucial to learning and memory (Yamada *et al.*, 2003). Indeed, Xu and colleagues (2000) confirmed that BDNF plays a significant role in LTP and that it acts through the TrkB receptor. Given that BDNF has been repeatedly shown to play a central role in producing the exercise-induced improvements in cognition, it is probable that changes in TrkB will accompany changes in BDNF in response to exercise. Widenfalk and colleagues (1999) demonstrated an exercise-induced increase in the expression of BDNF mRNA and TrkB mRNA in the hippocampus of spontaneously hypertensive rats (SHR). Rats were housed with a running wheel and different groups exercised for 5 or 6 weeks, the minimum distance run by rodents was 3km per night. In addition, Klintsova and

colleagues (2004) observed changes in TrkB protein expression, matching changes in BDNF protein expression, in the cerebellum of adult rats following forced exercise training (1, 3, 5 and 7 days). It is interesting that no exercise-induced effect on the expression of TrkB in the dentate gyrus of young animals was observed in the present studies. However, the distance run by rats did not exceed 1km per day, and it is important to highlight that the minimum distance run by rats in the study by Widenfalk and colleagues (1999) was 3km per night. In addition, Widenfalk and colleagues (1999) show that exercise-induced changes in BDNF and TrkB expression occur in a running length dependent manner, suggesting that the magnitude of an exercise-effect is dependent on the distance run. The shorter distance run by the animals in the current experiments may account for the different results obtained. The study by Klintsova and colleagues (2004) reported exercise-induced changes in BDNF and TrkB protein that were specific to the cerebellum. Although this result is important in demonstrating an exercise-effect on learning and shows that changes in BDNF and TrkB may occur simultaneously, the present studies focus on changes within the dentate gyrus, whole hippocampus and entorhinal cortex and so a direct comparison of results is not possible. The differences in study design and protein analysis may provide some explanation for the disparity between the present results and published data. On reflection, given the short duration of the exercise regime used in these experiments perhaps it would have been more valuable to analyse levels of TrkB activation. It is likely that exercise-induced changes in TrkB activation, accompanying an increase in BDNF concentration, would have been observed.

Activation of the p75 receptor is commonly associated with apoptotic cell signalling events (Lee *et al.*, 2001). Considering that exercise-induced enhancements of hippocampal function are repeatedly linked to cellular changes that promote neuronal cell survival, activity dependent plasticity and neurogenesis (Van der Borght *et al.*, 2007; van Praag *et al.*, 1999), it may be presumed that p75 expression would be unchanged or even reduced in response to exercise. The results from these studies show no exercise-effect on the expression of p75 in the dentate gyrus. Although there is very little research investigating the effect of exercise on p75, these results are in agreement with those of Widenfalk and colleagues (1999) who report no changes in p75 expression in the sensorimotor

cortex or the hippocampus following exercise in SHR rats.

Ying and colleagues (2002) demonstrate that BDNF-induced LTP in the adult hippocampus requires ERK activation. Specifically in the context of memory formation, BDNF signalling has been tightly linked with ERK1/2-dependent mechanisms and it has been suggested that BDNF-induced CREB activation is primarily mediated through the activation of ERK1/2 signalling (Alonso *et al.*, 2002b). It follows that ERK activation is likely to be involved in mediating the effects of exercise on hippocampal function. Indeed, research has shown that activation of the signalling molecule MAPK/ERK is significantly increased following exercise (Shen *et al.*, 2001). However, the experiments completed as part of these investigations show no evidence of an exercise-induced effect on ERK activation in the dentate gyrus. It is particularly important in the context of ERK activation to appreciate that neurotrophin signalling is extensive and signal transduction is not solely limited to ERK activation. Trk receptor activation leads to the activation of different signalling cascades including the MAPK, PI-3K and phospholipase C-γ pathways (Dan, 2004). Although ERK is definitely a key player concerning learning and memory (Adams & Sweatt, 2002) it is not unique as a signal transducer. Vaynman and colleagues (2006) revealed that 3 days of voluntary wheel running differentially regulates synaptic proteins associated to the function of BDNF. By extending this concept, it seems plausible that an exercise-induced increase in BDNF could be acting via different signalling pathways or multiple signalling pathways to mediate its effects on hippocampal function. In addition, Molteni and colleagues (2002), when investigating the effect of exercise on a variety of genes in the rat hippocampus, demonstrated that the CAMK system is more active during acute and chronic periods of exercise, where the MAPK/ERK system is more important during long-term exercise. The authors suggest that the temporal profile of gene expression delineates a mechanism by which specific molecular pathways are activated after exercise performance. Following from this, it is particularly relevant when considering the current data, to have an appreciation of the time-course of ERK induction. Ying and colleagues (2002) demonstrate a rapid phosphorylation of ERK following intra-hippocampal microinfusion of BDNF. A study completed by Skifter and colleagues (2002) demonstrates that ERK activation following permanent middle cerebral artery

occlusion (pMCAO), is progressively increased from 1hr post pMCAO peaking at 24hr. The increase in ERK activation in response to pMCAO was significant at 24hr. Collectively, these results illustrate the significance of the time-course of ERK induction and considering the study design used for the current experiments may explain why an exercise-induced increase in ERK activation was not observed here. It is speculated that an exercise-induced effect on ERK activation would more likely observed immediately post exercise.

Having discussed the results of these experiments and provided possible explanations where expected changes were not observed, it is equally important to acknowledge that the cellular changes mediating an exercise-induced effect on hippocampal function may involve other growth factors besides BDNF. Exercise-induced improvements in cognitive function have also been associated with IGF-1 (Carro *et al.*, 2001), VEGF (Ding *et al.*, 2006) and NGF (Ang *et al.*, 2003). Both Ang and colleagues (2003) and Neeper and colleagues (1996) have demonstrated a role for NGF in mediating the effects of exercise on hippocampal function. Interestingly, Neeper and colleagues (1996) observed an increase in hippocampal NGF mRNA after only 2 nights of voluntary wheel running. If NGF and other growth factors are contributing to exercise-induced effects on synaptic plasticity and learning, it suggests that an interplay of mechanisms exists to facilitate the exercise-induced effects on hippocampal function. Additionally, the time-course of induction for other growth factors may be different to that of BDNF, and will undoubtedly have implications for which intracellular cascades are activated and the timing of activation. An appreciation of this interplay of mechanisms is important when considering the biochemical changes analysed in these experiments. It is likely that the actions of other growth factors may also influence the biochemical mechanisms underlying the effects of exercise on hippocampal function.

3.4.3 Summary

The results of this study provide strong evidence of an exercise-related enhancement in hippocampal function in support of previous research. An exercise-induced improvement in LTP and recognition memory was demonstrated. No improvements in spatial learning were observed with acute

exercise. The positive exercise effect was associated with an increase in BDNF concentration within the dentate gyrus. For LTP experiments, an exercise-induced increase in BDNF concentration was observed in the hippocampus. For object recognition experiments, an exercise-induced increase in the concentration of BDNF in the hippocampus and entorhinal cortex was demonstrated. These results suggest that the cellular changes accompanying improvements in synaptic plasticity and learning may be region specific. No significant exercise-induced changes in the expression of proBDNF, the receptor sub-types TrkB and p75 or ERK activation in the dentate gyrus were identified. It is speculated that, in some instances, the timing of cellular modifications underlying an exercise-induced improvement in hippocampal function accounts for these results.

The results from these experiments support the notion that BDNF plays a central role in initiating the positive effects of exercise on cognitive function, however, it is speculated that different signalling pathways and signalling molecules may also be involved. It is proposed that 1-week of forced exercise results in selective enhancements of hippocampal function and that these may be dependent on an increased concentration of the neurotrophin BDNF.

Chapter 4

An investigation into the effect of acute exercise on LTP and spatial learning in young and aged animals. What are the underlying cellular mechanisms?

Chapter 4

An investigation into the effect of acute exercise on LTP and spatial learning in young and aged animals. What are the underlying cellular mechanisms?

4.1 Introduction

The natural aging process is accompanied by a decline in the function of many physiological systems. The hippocampus is a brain region that is vital to learning and memory and is particularly susceptible to the aging process. The aged brain exhibits both functional and structural deficits that negatively impact LTP, spatial learning and neurogenesis. Barnes and colleagues (2003) proposed that the age-related impairment in hippocampal-dependent forms of learning may be caused, in part, by altered mechanisms of synaptic plasticity within the hippocampus. One mechanism that has been associated with the age-related impairment in LTP is an impairment in BDNF signalling (Gooney *et al.*, 2004). Severe cognitive decline is seen with various neurodegenerative disorders such as dementia and Alzheimer's disease.

The data presented in the previous chapter demonstrate that acute exercise has positive effects on hippocampal function in young animals. In addition, these exercise-induced effects may be mediated by an up-regulation of BDNF in specific brain regions. This is supported by the literature that suggests BDNF is a key player in mediating the exercise-induced effects on cognitive function. In the context of neurodegenerative decline, an obvious question to ask is whether exercise provides a simple non-pharmacological intervention that may ameliorate this impairment in cognitive function. Interestingly, very few studies to date have explored the differential effects of age on exercise-induced changes within the brain.

There is evidence, from both human and animal studies, to suggest that cognitive function in older populations can be improved by exercise. Van Praag and colleagues (2005) demonstrated the potential for exercise to restore spatial learning and increase neurogenesis in aged animals. Nevertheless, there is no data to suggest whether an exercise program completed at different life stages has the same potential to improve hippocampal function. Radak and colleagues (2001) suggest that the impact of exercise on cognitive function and oxidative damage

within the rat brain is not markedly varied with age. However, Kim and colleagues (2004) observed more potent enhancing effects of treadmill exercise on cell proliferation in the dentate gyrus of 8-week-old animals, compared with 4-week-old and 62-week-old animals, suggesting that the enhancing effect of exercise on cell proliferation is dependent on age-status. There is an obvious need for further research to more clearly identify whether the exercise-induced effects on brain plasticity and learning are age-dependent. In addition, where exercise-induced improvements in cognitive function are observed, it is important to identify whether the cellular mechanisms mediating these effects are the same for young and aged animals.

The primary purpose of this study was to investigate the impact of an acute 7-day forced exercise program on LTP and spatial learning in differently aged populations of rats. The question "are the exercise-induced benefits to hippocampal function dependent on age?" will be addressed. Furthermore, the molecular mechanisms underlying these exercise-induced effects will be explored in an attempt to identify similarities and or differences between young, middle-aged and aged animals.

4.2 Materials and Methods

24 middle-aged rats and 24 aged rats were used for these experiments. Following familiarisation animals were equally divided into control and exercising groups. The exercise program for middle-aged animals was identical to that used for young animals; 7 days consecutive running at a speed of 17m/min for 60min (approximately 1km per day). Following some preliminary investigations, aged rats were run at a slower speed of 12.5m/min. To ensure the aged animals covered the same distance as the young and middle-aged groups, animals completed 4 x 20min treadmill sessions per day, with an inter-session rest period of 10min. They engaged in this regime for 7 consecutive days similar to the other groups. Control rats were placed on stationary treadmills for the same duration as age-matched exercising animals. Following completion of the exercise program animals were assessed for either LTP or spatial learning.

12 middle-aged animals (control n=6, exercised n=6) and 12 aged animals (control n=6, exercised n=6) were used for LTP experiments. Of the 12 middle-

aged animals, 2 died (one from each group) during the course of the experiments. 12 middle-aged and 12 aged animals (control n=6, exercised n=6 for all populations) were used for the MWM experiments. Within the middle-aged group, 5 animals died for unknown reasons during the course of the experiment leaving small group sizes at the end of the experiment (control n=5, exercised n=2).

Rats were sacrificed immediately after completion of the LTP and MWM experiments. The dentate gyrus was dissected (tissue was sliced or homogenised in Krebs solution) and stored as described previously (section 2.8). Tissue homogenate (dentate gyrus) was thawed and spun at 14,000 rpm for 5min to extract the supernatant for analysis of BDNF concentration by ELISA (section 2.10 and 2.10.1). Dentate gyrus samples were analysed for the expression of proBDNF, the neurotrophin receptor subtypes (TrkB and p75) and ERK by gel electrophoresis and western immunoblotting (sections 2.11, 2.12, 2.13 and 2.14).

When identifying cellular changes results from LTP and MWM experiments were analysed individually. Where no effect was observed age-matched data were pooled from control animals, and exercised animals separately, to identify if an exercise effect would be revealed with a larger sample size. Pooled data are presented unless otherwise specified.

4.3 Results

4.3.1 The effect of exercise on LTP

The effect of an acute 7-day forced exercise program on LTP in young, middle-aged and aged rats was assessed. Results for young animals are shown in Chapter 3; Figure 3.1a & b. No exercise-induced enhancement in LTP was observed in middle-aged animals. Values for mean % EPSP during the last 5min of the experiment were 117.2 ± 5.9 and 144.5 ± 14.3 % for control and exercising groups respectively (Figure 4.1b). Two animals (one from each group) died during the experiments leaving smaller group sizes for statistical analysis. For aged animals (Figure 4.2a & b) statistical analysis revealed marked differences between groups. Analysis by 2-way ANOVA revealed a significant exercise-induced improvement in LTP ($p<0.001$). There was no interaction between groups that suggests there was an exercise-induced effect on both LTP induction and the

ability to sustain LTP. There was also a significant difference in the mean % EPSP slope ($p<0.001$, 112.6 ± 3.7 and 141.7 ± 3.6 % for control and exercising animals respectively).

To identify whether there was a differential effect of age on the potential for exercise to enhance LTP a comparison of all groups was completed. Statistical analysis by 2-way ANOVA of the mean % EPSP (± SEM) during the last 5min of the experiment for all groups was completed. Analysis revealed an exercise effect in aged animals ($p<0.01$).

4.3.1.1 The effect of age on LTP

Figure 4.4a & b show data from control animals (young, middle-aged and aged groups). Statistical analysis by 2-way ANOVA post tetanus did not show any evidence of an age-related impairment in LTP. There was no significant difference between young (115.1 ± 2.49 %), middle-aged (117.2 ± 5.9 %) and aged (112.6 ± 3.7 %) animals for mean % EPSP during the last 5min of the experiment.

Rat	Young % EPSP	Middle-aged % EPSP	Aged % EPSP
1	118.21	91.68	103.97
2	169.59	143.45	113.23
3	105.84	136.94	126.84
4	112.67	152.02	118.09
5	114.75	109.35	107.05
6	92.01	119.25	109.88

Table 4.1 % EPSP values for young, middle aged and aged control rats immediately post HFS are presented. These data give an indication of the ability to induce LTP. Rats that did not show increased EPSP following HFS are highlighted in red.

Table 4.1 shows % EPSP data for all age groups immediately post HFS. In young animals 1/3 of the sample did not show increased EPSP post HFS, in middle-aged animals 1/3 of the group did not show increased EPSP and in aged animals 1/2 of the group did not show increased EPSP. These data suggest that there is

variability in the ability to induce LTP in all age groups.

4.3.2 Effect of exercise on spatial learning

The MWM task was used to assess spatial learning in young, middle-aged and aged populations of animals. There was no exercise-induced improvement in spatial learning in any population of rats; data were analysed by 2-way ANOVA to determine if there was a significant effect of day or exercise on escape latency. Results for young animals are shown in Chapter 3; Figure 3.3a. For middle-aged animals (Figure 4.5a) on day 1 of the task mean values for escape latency were 52 ± 2 and 57 ± 3 secs for control and exercised groups respectively. On day 5 of the task mean values for escape latency were 41 ± 6 and 40 ± 6 secs for control and exercised groups respectively. Statistical analysis was not valid for middle-aged exercising animals (n=2), ideally these experiments should be repeated to identify if there is an exercise-induced effect on spatial learning in this population. For aged animals (Figure 4.5b) mean values for escape latency on day 1 of the task were 54 ± 2 and 51 ± 4 secs for control and exercised groups respectively. On day 5 of the task, mean values for escape latency were 44 ± 5 and 37 ± 9 secs for control and exercised groups respectively; no significant differences were observed.

Data from control and exercised groups were analysed separately to identify if there was an effect of day on escape latency; analysis was completed by 1-way ANOVA. There was no effect of day on escape latency in any of the aged groups that suggests no group learnt the task.

4.3.2.1 Effect of exercise on probe test performance

There was no effect of exercise on performance in the probe test for any group of animals. The results for young rats are shown in Chapter 3; Figure 3.3b, c & d. With such a small sample size for middle-aged exercising animals statistical analysis is not valid. For aged animals, analysis of the percentage time swimming in the NW quadrant compared with all other pool quadrants revealed no preference for the NW quadrant (pool quadrant where the escape platform had been located). This result confirms that animals had not learnt the task. Percentage time for middle-aged control animals was 24.9 ± 2.5, 27.5 ± 3.3, 19.1 ± 3.3 and

28.5 ± 3.3 % for SW, NE, NW and SE respectively. Percentage time for middle-aged exercised animals was 26.5 ± 1.2, 23.3 ± 2.7, 24.8 ± 5.8 and 25.3 ± 9.7 % for SW, NE, NW and SE respectively. Percentage time for aged control animals was 30.9 ± 3.2, 20.6 ± 2.1, 25.6 ± 4.8 and 22.9 ± 2.7 % for SW, NE, NW and SE respectively. Percentage time for aged exercised animals was 32.9 ± 3.8, 21.8 ± 1.2, 24.5 ± 4.6 and 20.7 ± 2.0 % for SW, NE, NW and SE respectively. Analysis of the time spent swimming in the NW quadrant for the probe test revealed no effect of exercise on probe trial performance for aged animals (Figure 4.5d). Time in the NW quadrant was 11.4 ± 2.01 and 14.9 ± 3.5 secs for control and exercising middle-aged animals respectively. For aged animals time in the NW quadrant was 15.4 ± 2.9 and 14.7 ± 2.8 secs for control and exercising animals respectively. Cumulatively these results support the escape latency data showing no effect of exercise on spatial learning.

4.3.2.2 Effect of age on spatial learning

To identify whether there was an age-related effect on spatial learning MWM performance of young, middle-aged and aged control animals was compared. Analysis by 2-way ANOVA was completed to identify if there was an effect of day or age on escape latency. This analysis did not reveal an effect of age but did reveal an effect of day on escape latency in young animals. Data from young, middle-aged and aged control rats, on separate days, were also analysed by 1-way ANOVA. This analysis revealed significant differences in escape latency between age groups on day 2, 3 and 5 and provides evidence of an age-related decline in spatial learning (Figure 4.6). On day 2 mean values for escape latency were 33.6 ± 5.1, 51.3 ± 5.3 and 52.2 ± 4.5 secs for young, middle-aged and aged animals respectively ($p<0.05$). On day 3 mean values for escape latency were 29.3 ± 5.7, 48.7 ± 4.8 and 49.4 ± 4.0 secs for young, middle-aged and aged populations respectively ($p<0.05$). On day 5 of the task there was a significant difference ($p<0.05$) in the mean escape latency of young (22.8 ± 6.7 secs) and aged (44.2 ± 5.4 secs) control animals.

4.3.2.3 Effect of age on MWM task performance; swim speed and pathlength

In view of the result showing an age-related impairment in spatial learning,

assessed by analysis of escape latency, it was important to consider whether swim speed may have contributed to the differences observed between young, middle-aged and aged animals. There were significant differences in both swim speed (cm/secs) and pathlength (cm) when comparing young animals with middle-aged or aged animals (Figure 4.7a & b). There were no significant differences between middle-aged and aged groups for swim speed or pathlength. Data for all age groups are presented in Table 4.2, 4.3 and 4.4.

YOUNG ANIMALS	SWIM SPEED (CM/SECS)	PATHLENGTH (CM)
DAY 1	15.2 ± 1.4	680.7 ± 41.7
DAY 2	20.7 ± 1.1	683.7 ± 100.1
DAY 3	19.5 ± 1.6	506.3 ± 71.2
DAY 4	17.2 ± 1.0	484.0 ± 90.1
DAY 5	16.7 ± 1.3	354.3 ± 76.2

Table 4.2 Results for young animals for swim speed and pathlength during the MWM task

MIDDLE-AGED ANIMALS	SWIM SPEED (CM/SECS)	PATHLENGTH (CM)
DAY 1	26.2 ± 1.1	1399.8 ± 53.8
DAY 2	27.4 ± 1.6	1437.0 ± 201.8
DAY 3	28.0 ± 2.4	1334.0 ± 126.9
DAY 4	27.2 ± 1.6	1201.8 ±136.8
DAY 5	28.0 ± 1.3	1138.8 ± 145.5

Table 4.3 Results for middle-aged animals for swim speed and pathlength during the MWM task

AGED ANIMALS	SWIM SPEED (CM/SECS)	PATHLENGTH (CM)
DAY 1	21.7 ± 1.5	1009.4 ± 89.9
DAY 2	25.6 ± 1.4	1206.6 ± 121.3
DAY 3	26.3 ± 1.5	1198.2 ± 142.9
DAY 4	27.3 ± 1.5	1051.8 ± 159.1
DAY 5	26.6 ± 0.9	1040.0 ± 161.5

Table 4.4 Results for aged animals for swim speed and pathlength during the MWM task

4.3.3 Effect of exercise on BDNF concentration

The impact of short-term exercise on the concentration of BDNF in the dentate gyrus was investigated in young, middle-aged and aged animals. An exercise-induced increase in BDNF following acute exercise was observed in young rodents (results are shown in Chapter 3; Figure 3.4a). However, there was no exercise-induced effect on BDNF concentration in middle-aged or aged animals. For middle-aged animals BDNF concentration in the dentate gyrus was 0.16 ± 0.06 and 0.15 ± 0.06 ng/mg for control and exercised animals respectively (Figure 4.8a). For aged animals BDNF concentration in the dentate gyrus was 0.14 ± 0.05 and 0.12 ± 0.03 ng/mg for control and exercising animals respectively (Figure 4.8b).

4.3.3.1 Effect of age on BDNF concentration

The effect of age on BDNF concentration in the dentate gyrus was investigated. No age-related effects were observed; there was no difference in the concentration of BDNF in the dentate gyrus of young, middle-aged and aged animals. BDNF concentration was 0.18 ± 0.03, 0.16 ± 0.06 and 0.14 ± 0.05 ng/mg for young, middle-aged and aged animals respectively (Figure 4.8c).

4.3.4 Effect of exercise on proBDNF expression

The effect of an acute forced exercise program on the expression of proBDNF in

the dentate gyrus of young, middle-aged and aged animals was assessed. There was no significant effect of exercise on proBDNF expression in young animals (Chapter 3; Figure 3.5). Similarly, there were no exercise-induced effects on proBDNF expression in middle aged or aged animals (Figure 4.9a & b). The expression of proBDNF was 0.36 ± 0.07 and 0.61 ± 0.15 proBDNF/β-actin for middle-aged control and exercising animals respectively (Figure 4.9a). For aged animals (Figure 4.9b) proBDNF expression in the dentate gyrus was 0.24 ± 0.06 and 0.31 ± 0.10 proBDNF/β-actin for control and exercising animals respectively.

4.3.4.1 Effect of age on proBDNF expression

The effect of age on the expression of proBDNF in the dentate gyrus of young, middle-aged and aged animals was analysed. An age-related decrease in the expression of proBDNF was observed. The expression of proBDNF was 0.82 ± 0.12, 0.36 ± 0.07 and 0.24 ± 0.06 proBDNF/β-actin for young, middle-aged and aged animals respectively (Figure 4.9c). Statistical analysis using a 1-way ANOVA revealed significant changes; $p<0.01$ for young vs middle-aged and $p<0.001$ for young vs aged animals.

4.3.5 The effect of exercise on TrkB expression

The effect of an acute forced exercise program on the expression of TrkB in the dentate gyrus of young, middle-aged and aged animals was investigated. There was no exercise effect on TrkB expression in young animals (Chapter 3; Figure 3.6a). Similarly, there was no effect of acute exercise on TrkB expression in middle-aged animals. TrkB expression was 0.67 ± 0.11 and 0.68 ± 0.19 TrkB/β-actin for control and exercising middle-aged animals respectively (Figure 4.10a). There was an exercise-induced increase in TrkB expression in aged animals ($p<0.05$). TrkB expression in the dentate gyrus was 0.82 ± 0.14 and 1.45 ± 0.22 TrkB/β-actin for control and exercising aged animals respectively (Figure 4.10b).

4.3.5.1 The effect of age on TrkB expression

The effect of age on TrkB expression in the dentate gyrus of young, middle-aged and aged control animals was assessed. Statistical analysis was completed using a 1-way ANOVA. Although there was no significant effect of age on the expression

of TrkB, there may a trend for an age-related increase in this receptor (Figure 4.10c). TrkB expression was 0.54 ± 0.16, 0.67 ± 0.11 and 0.82 ± 0.14 TrkB/β-actin for young, middle-aged and aged animals respectively.

4.3.6 The effect of exercise on p75 expression

The effect of acute exercise on p75 expression in the dentate gyrus of young, middle-aged and aged animals was investigated. There was no exercise-induced effect on p75 expression in young animals (Chapter 3; Figure 3.6b). Similarly, no effect of exercise was observed with middle-aged animals (Figure 4.11a). Expression of p75 was 0.23 ± 0.08 and 0.15 ± 0.04 p75/β-actin for control and exercising animals respectively. Interestingly, there was evidence of an exercise-induced decrease in p75 expression in the dentate gyrus for aged animals ($p<0.05$). Expression of p75 was 0.19 ± 0.04 and 0.08 ± 0.03 p75/β-actin for control and exercising animals respectively (Figure 4.11b).

4.3.6.1 The effect of age on p75 expression

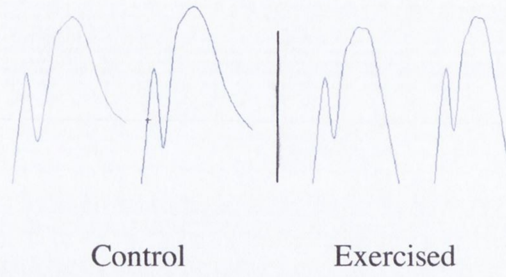
The effect of age on p75 expression in the dentate gyrus of young, middle-aged and aged control animals was assessed. Statistical analysis was completed using a 1-way ANOVA. There was no effect of age on the expression of p75 (Figure 4.11c). Expression of p75 was 0.16 ± 0.05, 0.23 ± 0.08 and 0.19 ± 0.04 p75/β-actin for young, middle-aged and aged animals respectively.

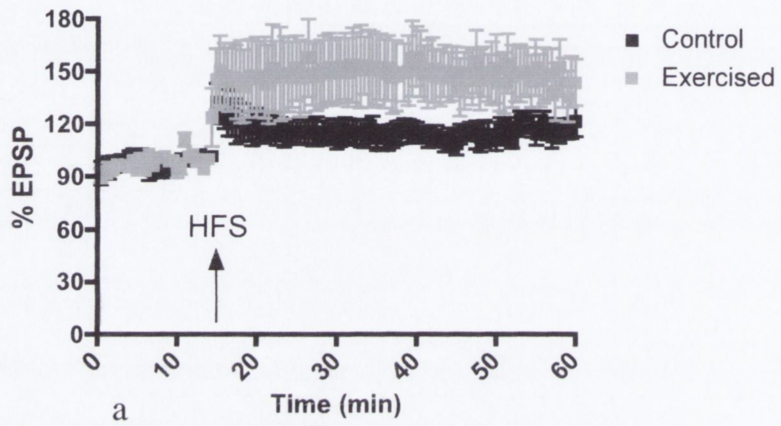
4.3.7 The effect of exercise on ERK activation

The effect of acute exercise on ERK activation in the dentate gyrus of young, middle-aged and aged animals was investigated. No exercise-induced effect was observed in any age group. Results for ERK activation in young animals are shown in Chapter 3; Figure 3.5. ERK activation for middle-aged animals was 0.22 ± 0.05 and 0.23 ± 0.07 p44/tERK (arbitrary units) for control and exercising animals respectively (Figure 4.12a). For aged animals, ERK activation was 0.23 ± 0.05 and 0.25 ± 0.04 p44/tERK (arbitrary units) for control and exercising animals respectively (Figure 4.12b).

4.3.7.1 The effect of age on ERK activation

To assess for an effect of age on ERK activation in the dentate gyrus young, middle-aged and aged control animals were compared. Statistical analysis was completed using a 1-way ANOVA. There appears to be a trend for reduced ERK activation in older animals but these differences were not significant ($p=0.0607$). ERK activation was 0.23 ± 0.02, 0.22 ± 0.05 and 0.23 ± 0.05 p44/tERK (arbitrary units) for young, middle-aged and aged animals respectively (Figure 4.12c).





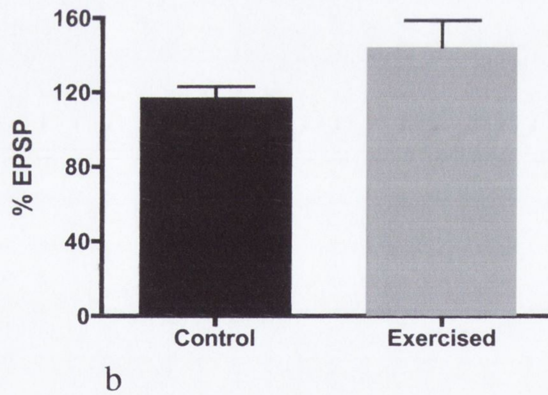


Figure 4.1a & b The effect of exercise on LTP expression in middle-aged animals

Figure 4.1a shows mean data (± SEM) for each test shock delivered pre and post HFS; data were analysed by 2-way ANOVA. Figure 4.1b shows mean values (± SEM) for % EPSP during the last 5min of the experiment following tetanic stimulation for control (n=5) and exercising (n=5) animals; an unpaired Student's t-test was used for statistical analysis. There was no significant effect of exercise on LTP in middle-aged animals.

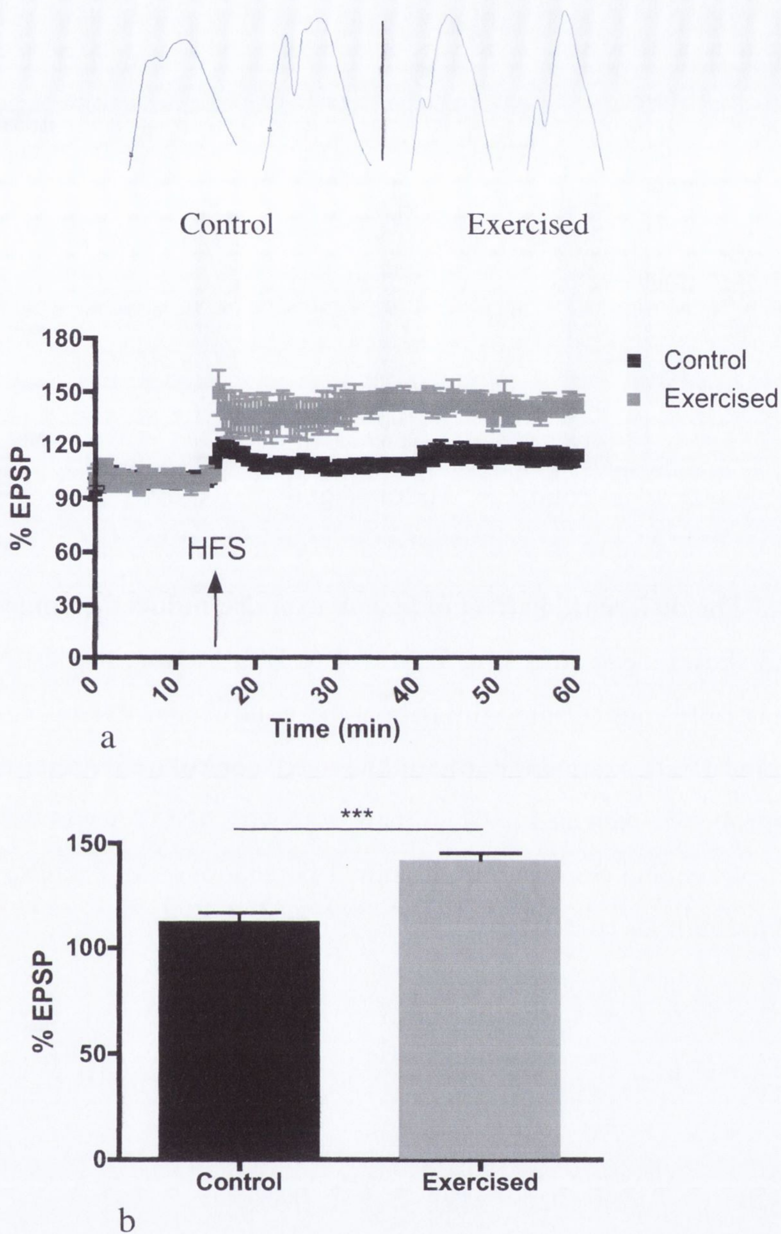


Figure 4.2a & b The effect of exercise on LTP expression in aged animals

Figure 4.2a shows mean data (± SEM) for each test shock delivered pre and post HFS. There was a significant effect of exercise when data were analysed by 2-way ANOVA ($p<0.001$). Figure 4.2b shows mean values (± SEM) for % EPSP during the last 5min of the experiment following tetanic stimulation for control (n=6) and exercising (n=6) animals; an unpaired Student's t-test was used for statistical analysis. There was an exercise-induced enhancement in the ability to sustain LTP in aged animals ($p<0.001$).

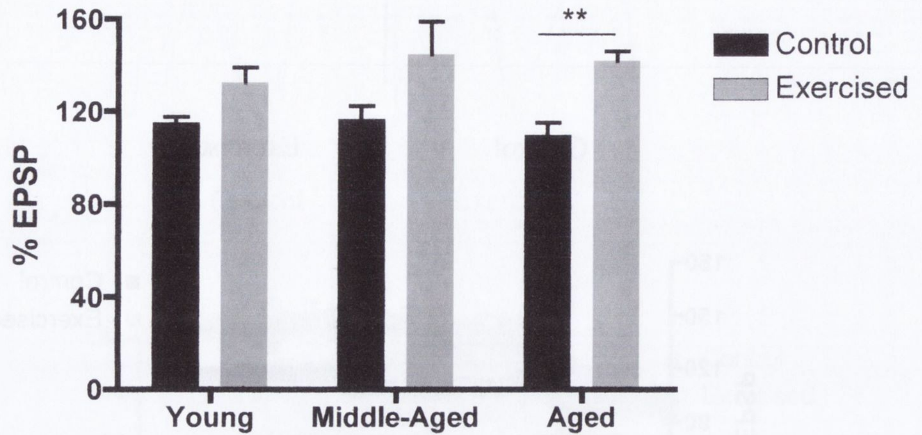


Figure 4.3 The differential effect of age on exercise-induced changes in LTP

Figure 4.3 shows mean values (± SEM) for % EPSP during the last 5min of the experiment following tetanic stimulation for control and exercising groups of young (control n=6, exercised n=6), middle-aged (control n=5, exercised n=5) and aged (control n=6, exercised n=6) animals. A 2-way ANOVA was used to assess the effect of age and exercise on all animal populations. An exercise effect was observed in aged rats ($p<0.01$).

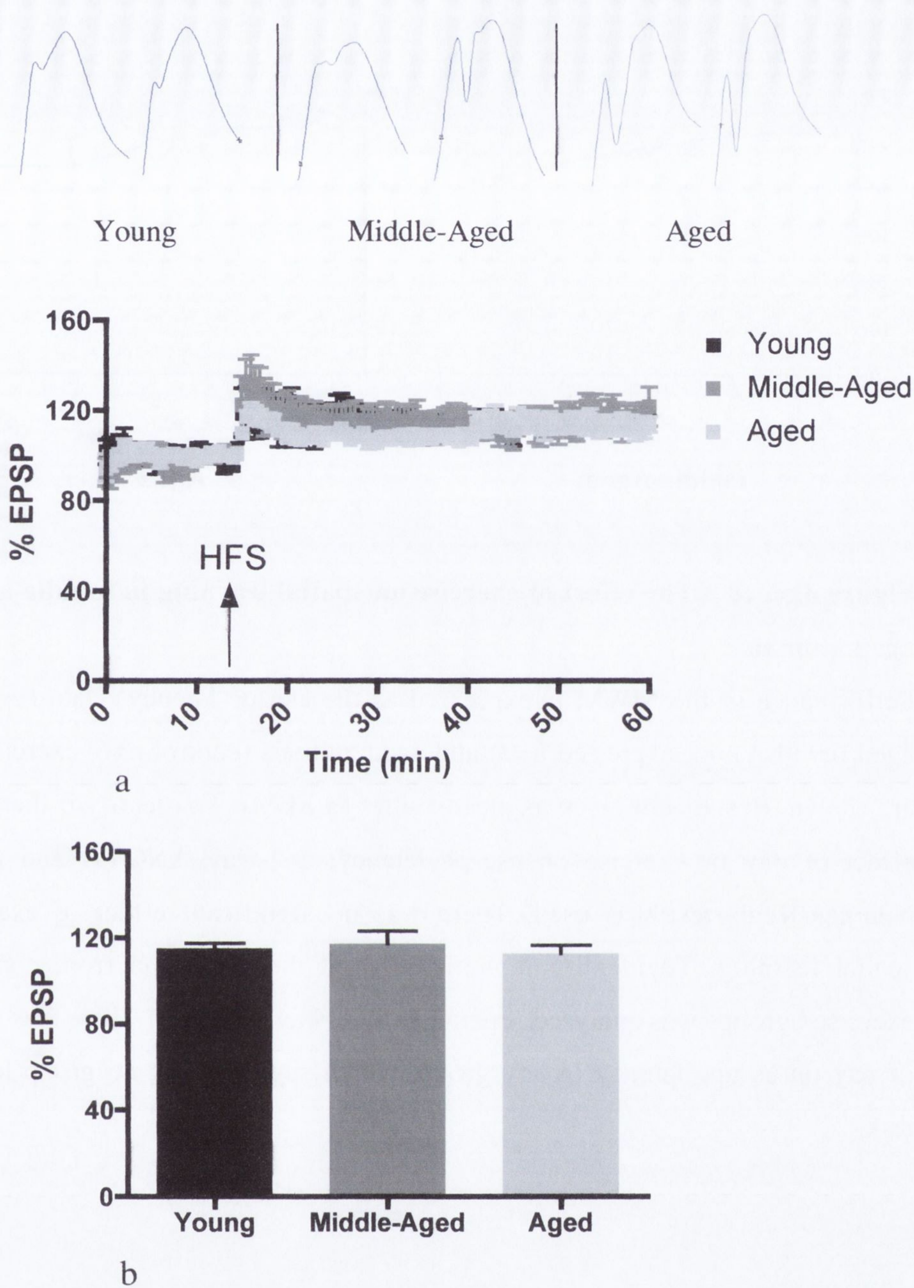


Figure 4.4a & b The effect of age on LTP

Figure 4.4a shows mean data (± SEM) for each test shock delivered pre and post HFS for young (n=6), middle-aged (n=5) and aged (n=6) control animals. Mean values (± SEM) for % EPSP during the last 5min of the experiment following tetanic stimulation are shown in Figure 4.4b. A 1- or 2-way ANOVA was used for statistical analysis. There was no significant effect of age on LTP.

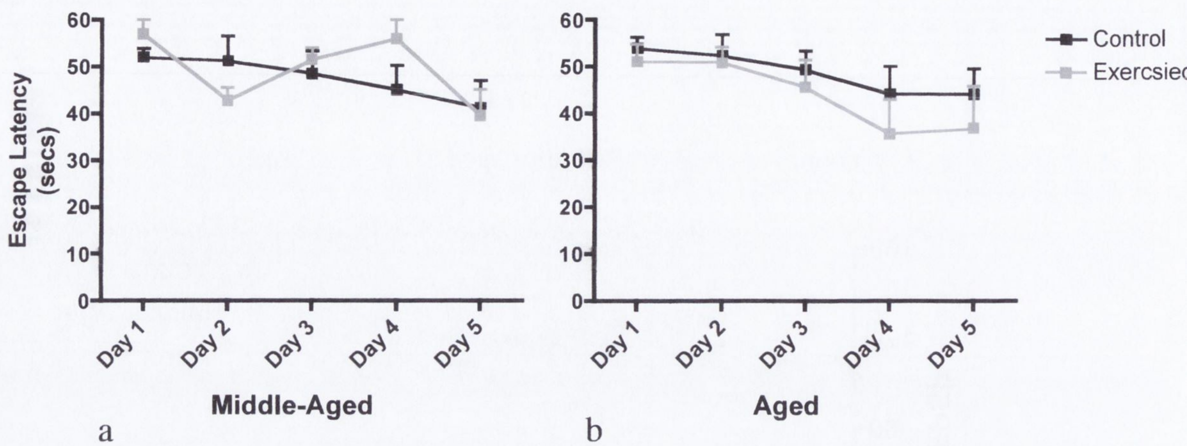


Figure 4.5a & b The effect of exercise on spatial learning in middle-aged and aged animals

Performance in the MWM is expressed as the escape latency. Data for middle-aged (control n=5, exercised n=2) and aged animals (control n=6, exercised n=6) are shown. Results are given as mean values (± SEM). To identify if there was an effect of day or exercise on escape latency, a 2-way ANOVA and post hoc Neuman-Keuls test was used. There was no significant effect of exercise on spatial learning. To identify if animals learnt the task, data from control and exercised groups was analysed separately by 1-way ANOVA. There was no effect of day on escape latency in any group, which suggests that no group learnt the task.

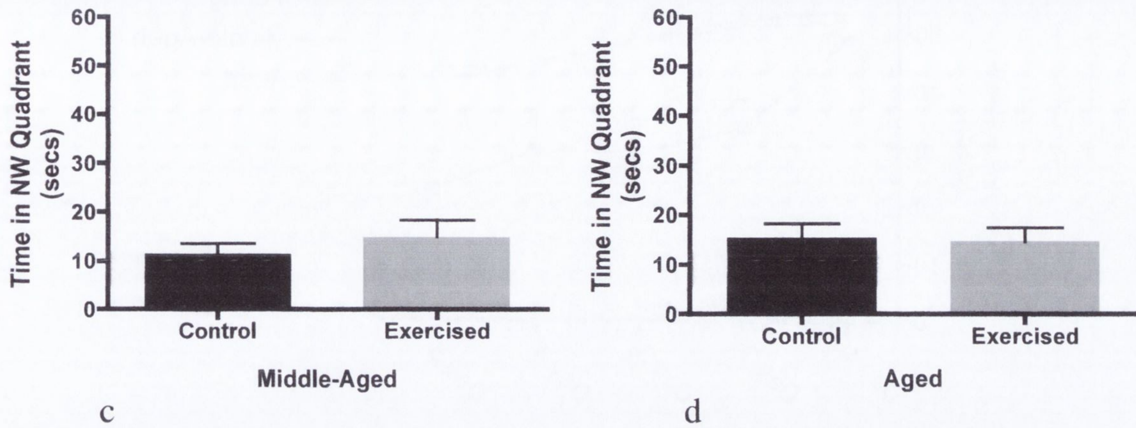


Figure 4.5c & d The effect of exercise on performance in the probe trial for middle-aged and aged animals

Time spent in the NW pool quadrant during the probe test for middle-aged (control n=5, exercised n=2) and aged (control n=6, exercised n=6) animals is shown. Results are given as mean values (± SEM); for statistical analysis an unpaired Students' t-test was used. There was no significant effect of exercise on probe trial performance. This result supports the analysis of escape latency data that showed no effect of exercise on spatial learning.

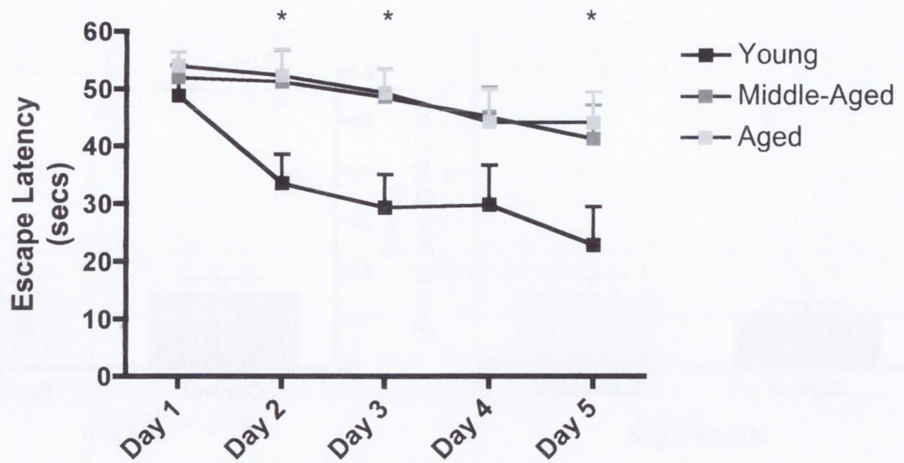


Figure 4.6 The effect of age on spatial learning

Performance in the MWM is expressed as the escape latency. Mean values (± SEM) for control animals in young (n=6), middle-aged (n=5) and aged (n=6) populations are shown. Analysis by 1-way ANOVA of data from young, middle-aged and aged animals, on separate days, revealed significant differences between age groups. On day 2 and day 3 of the task middle-aged and aged animals were significantly slower ($p<0.05$) at finding the escape platform than young animals. On day 5 of the task there was a significant difference ($p<0.05$) in the mean escape latency of young and aged control animals. This suggests that spatial learning is impaired with age.

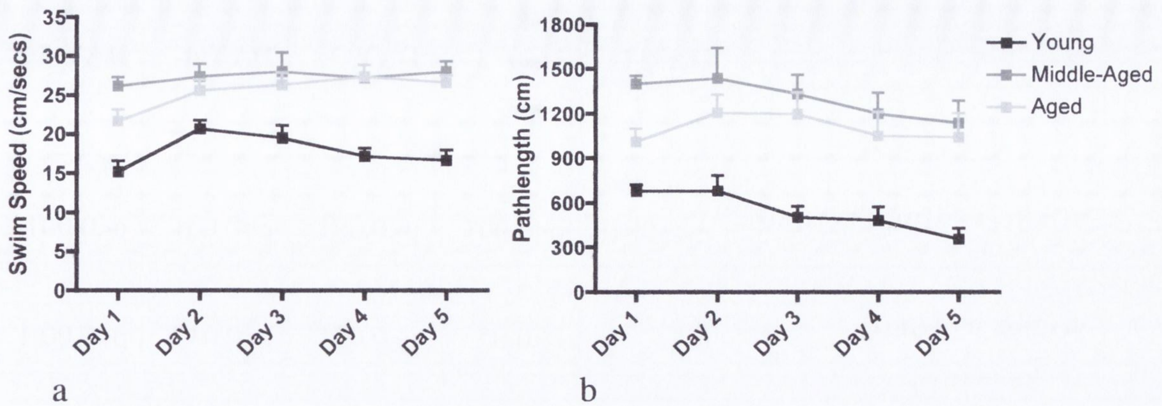


Figure 4.7a & b The differences between young, middle-aged and aged animals for swim speed and pathlength during the MWM task

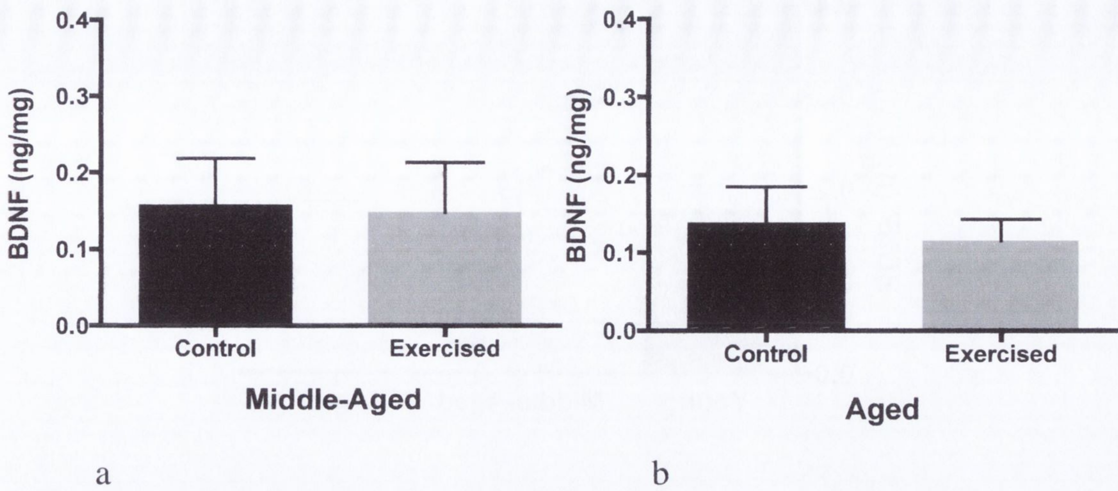
Results are given as mean values (± SEM). For statistical analysis a 2-way ANOVA with post hoc Neuman-Keuls was used. There was a significant effect of age on swim speed and pathlength. Young animals (n=6) were faster swimmers than middle-aged (n=5) and aged (n=6) animals throughout the MWM task and also had a significantly shorter pathlength than middle-aged and aged animals (Table 4.5a & b). There were no significant differences between middle-aged and aged animals for swim speed or pathlength.

SWIM SPEED (CM/SECS)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
YOUNG VS MIDDLE-AGED	p<0.001	p<0.01	p<0.001	p<0.001	p<0.001
YOUNG VS AGED	p<0.01	n/s	p<0.01	p<0.001	p<0.001

Table 4.5a Results for statistical analysis of the effect of age on swim speed during the MWM task

PATHLENGTH (CM)	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
YOUNG VS MIDDLE-AGED	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
YOUNG VS AGED	n/s	P<0.05	p<0.001	p<0.01	p<0.00 1

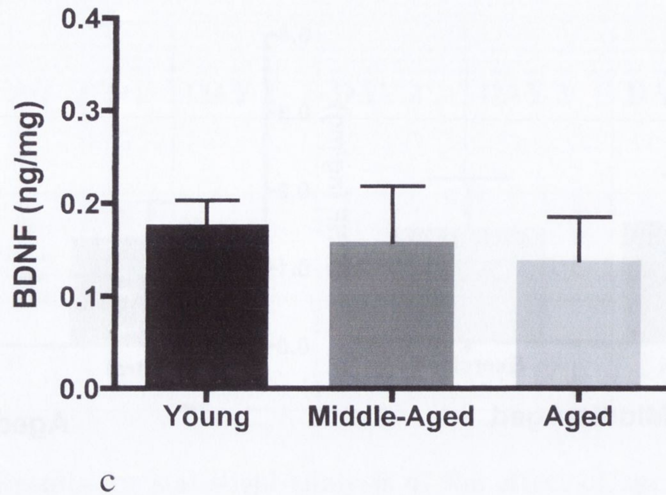
Table 4.5b Results for statistical analysis of the effect of age on pathlength during the MWM task



a b

Figure 4.8a & b The effect of exercise on the concentration of BDNF

Results are expressed as mean values (± SEM); unpaired Student's *t*-tests were used for statistical analysis. There was no exercise-effect on the concentration of BDNF in either middle-aged (control n=10, exercised n=7) or aged (control n=12, exercised n=12) animals.



c

Figure 4.8c The effect of age on BDNF concentration

Results for BDNF concentration in the dentate gyrus of young (n=12), middle-aged (n=10) and aged (n=12) control animals are shown. Results are expressed as mean values (± SEM); a 1-way ANOVA was used for statistical analysis. There were no differences in BDNF concentration when comparing young, middle-aged and aged animals. This result suggests that there is no age effect on BDNF concentration.

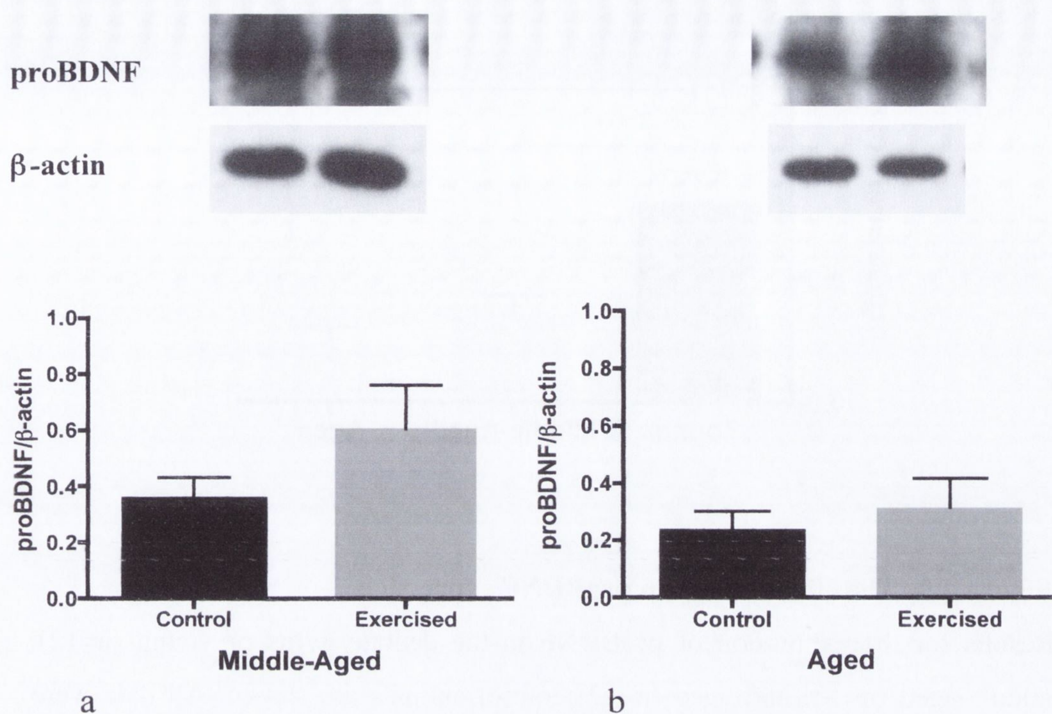


Figure 4.9a & b The effect of exercise on the expression of proBDNF

Results are expressed as mean values (± SEM); unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no effect of exercise on the expression of proBDNF in the dentate gyrus for middle-aged (control n=10, exercised n=7) or aged (control n=12, exercised n=12) animals.

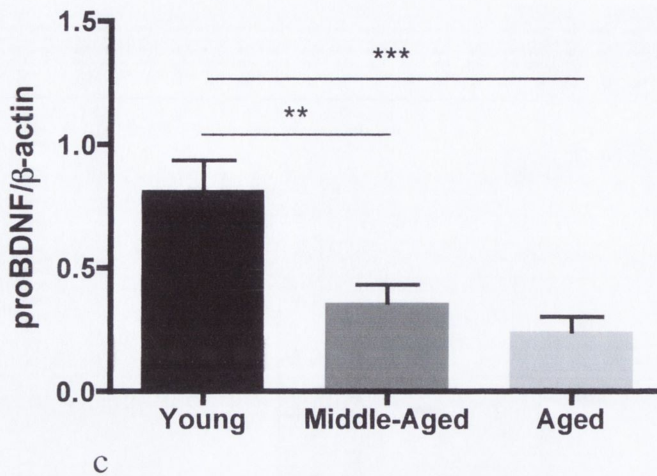


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Figure 4.9c The effect of age on proBDNF expression

Results for the expression of proBDNF in the dentate gyrus of young (n=12), middle-aged (n=10) and aged (n=12) control animals are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Statistical analysis was completed using a 1-way ANOVA; $p<0.01$ for young and middle-aged and $p<0.001$ for young and aged animals. This result suggests an age-related decrease in proBDNF expression.

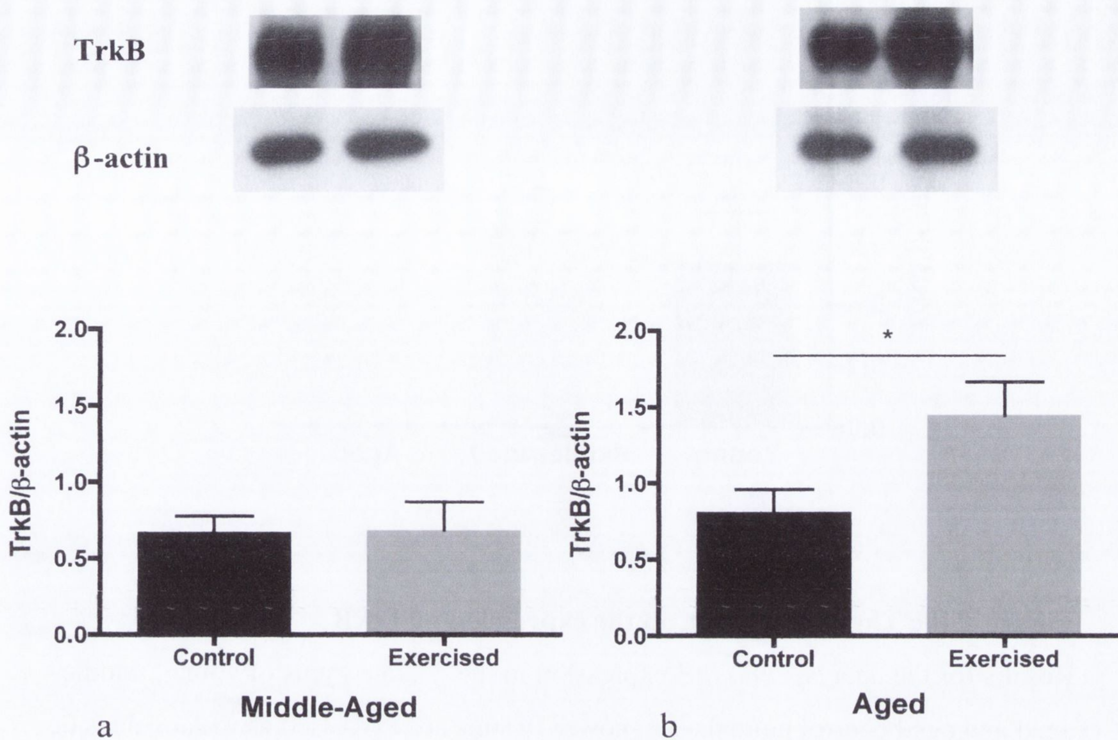

Figure 4.10a & b The effect of exercise on the expression of TrkB

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no exercise-induced effect on the expression of TrkB in the dentate gyrus of middle-aged animals (control n=10, exercised n=7). An exercise-induced increase in TrkB expression in the dentate gyrus of aged animals was observed ($p<0.05$, control n=12, exercised n=12).

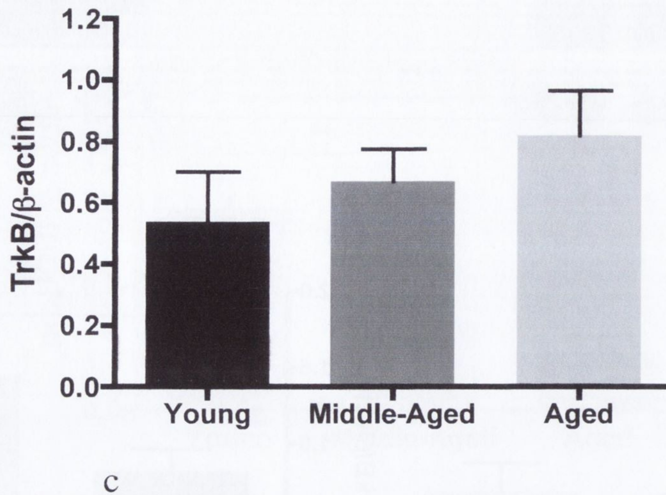

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Figure 4.10c The effect of age on the expression of TrkB

Results for the analysis of TrkB expression in the dentate gyrus of young, middle-aged and aged control animals are shown. Results are expressed as mean values (± SEM); a 1-way ANOVA was used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no difference in the concentration of TrkB in the dentate gyrus for young (n=12), middle-aged (n=10) and aged (n=12) animals. This suggests that there is no age-related effect on the expression of TrkB.

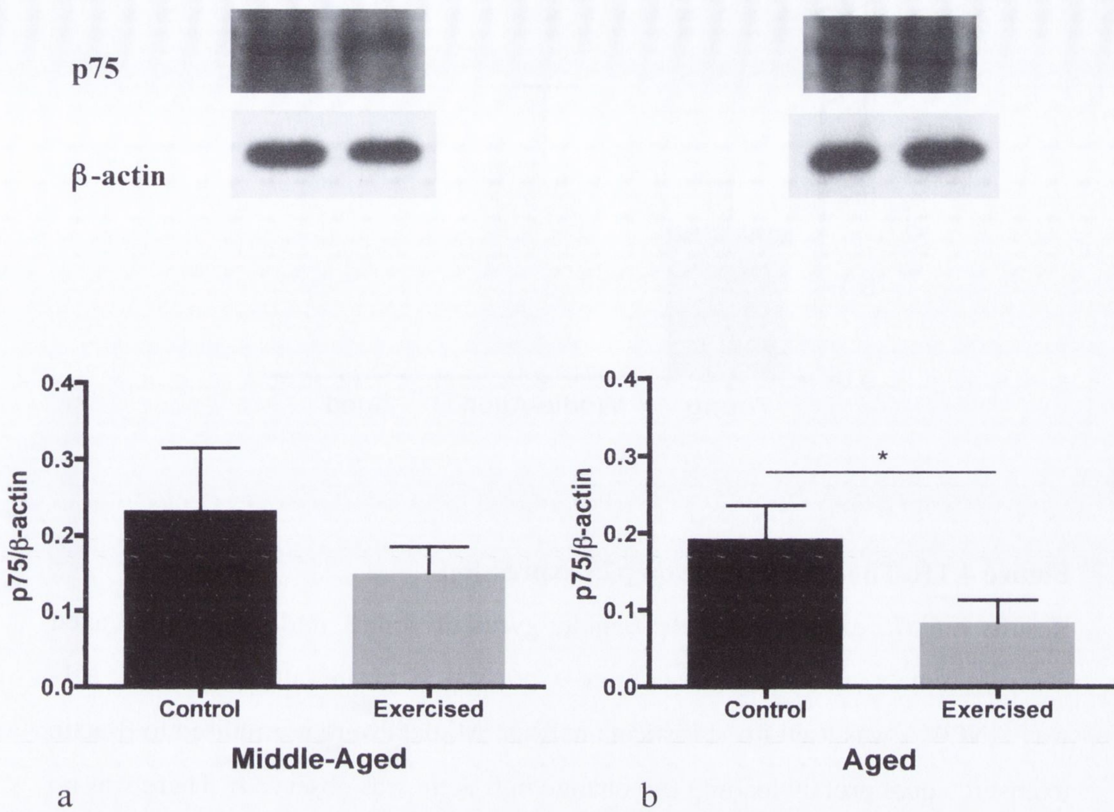

Figure 4.11a & b The effect of exercise on the expression of p75

Results are expressed as mean values (± SEM); unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no exercise-induced effect on p75 expression in the dentate gyrus of middle-aged animals (control n=10, exercised n=7). An exercise-induced decrease in p75 expression in the dentate gyrus for aged animals was observed ($p<0.05$, control n=12, exercised n=12).

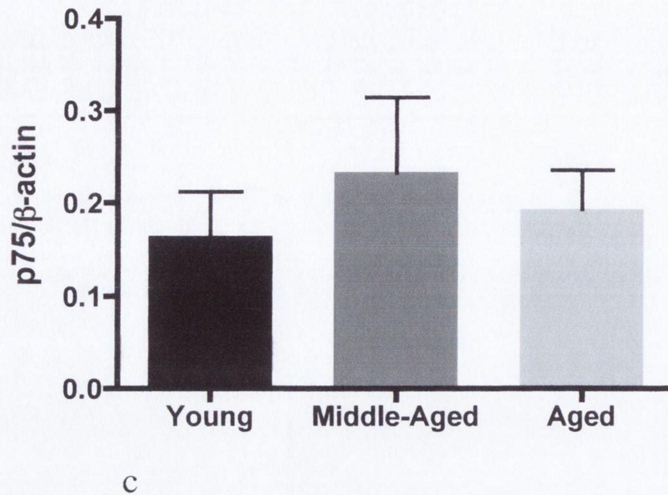

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Figure 4.11c The effect of age on p75 expression

Results for p75 expression in the dentate gyrus of young, middle-aged and aged control animals are shown. Results are expressed as mean values (± SEM); a 1-way ANOVA was used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no difference in the expression of p75 in the dentate gyrus when comparing young (n=12), middle-aged (n=10) and aged (n=12) animals. This result suggests that there is no effect of age on p75 expression.

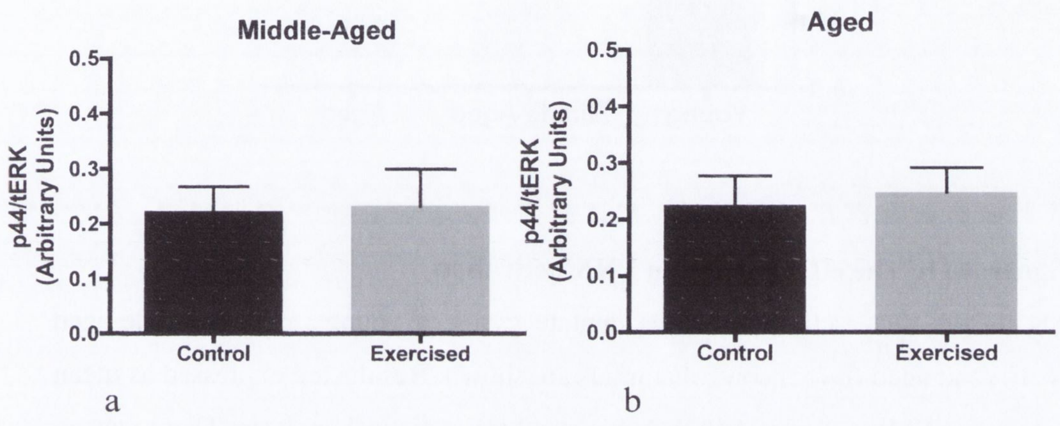
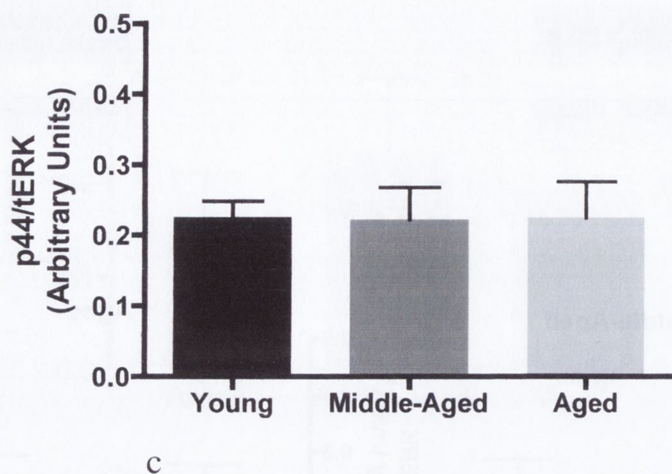


Figure 4.11a & b The effect of exercise on ERK activation

Results are expressed as mean values (± SEM); unpaired Student's *t*-tests were used for statistical analysis. There was no exercise-induced effect on ERK activation in the dentate gyrus of middle-aged (control n=10, exercised n=7) or aged (control n=12, exercised n=12) animals.



c

Figure 4.11c The effect of age on ERK activation

Results for ERK activation in the dentate gyrus of young (n=12), middle-aged (n=10) and aged (n=12) control animals are shown. Results are expressed as mean values (± SEM); a 1-way ANOVA was used for statistical analysis. There was no age-related effect on ERK activation.

4.4 Discussion

The main aim of this study was to investigate the effect of an acute 7-day exercise program on LTP and spatial learning in young, middle-aged and aged animals, and by these investigations identify whether there was a differential effect of age on exercise-induced changes in hippocampal function. In addition, biochemical analysis was completed to determine what cellular mechanisms may be mediating an exercise-effect in young, middle-aged and aged animals. Finally, analysis was completed to determine whether there was an effect of age on hippocampal function and other measured cellular parameters.

An exercise-induced enhancement in LTP in young and aged animals was observed. Spatial learning, a hippocampal-dependent form of learning, was not improved by exercise in young, middle-aged or aged animals. Exercise-induced changes were associated with an increased concentration of BDNF in young animals. The exercise-induced effects on hippocampal function in aged animals were associated with elevated TrkB and reduced p75 in the dentate gyrus. Considering age-related changes in hippocampal function, there was no marked impairment of LTP with age but an age-related decline in spatial learning was demonstrated. An age-related decrease in proBDNF expression in the dentate gyrus was also observed.

4.4.1 The effect of exercise on LTP

To date, there have been few studies investigating the impact of exercise on cognitive function in middle-aged and aged populations. The results of these investigations clearly show that acute exercise has the potential to enhance LTP in aged animals. Data analysis revealed that acute exercise produced an enhancement in both LTP induction and the ability to sustain LTP. Although the results for middle-aged animals suggest a similar trend for an exercise-induced enhancement in LTP, no significant differences were identified. There were difficulties experienced with this group of animals and a number of rats from both LTP and MWM experiments died for unknown reasons during the experimental period. It is likely that the reduced sample size influenced statistical analysis of data from these groups. Despite this, data indicates an exercise-induced improvement in LTP in aged animals and supports van Praag and colleagues

(2005) who demonstrated an exercise-induced improvement in neural plasticity in aged rodents. Van Praag and colleagues (2005) showed that a voluntary exercise program lasting 45 days produced improvements in hippocampal neurogenesis. In the current studies, 7 days treadmill running produced a marked improvement in LTP which is promising as it suggests even short periods of exercise completed by aged rats may induce cellular modifications, such as an improvement in synaptic strength.

There is conflicting evidence within the literature on the differential effect of age on the benefits of exercise to brain function. Radak and colleagues (2001), by investigating the effects of a 9-week swimming exercise regime on cognitive function in middle-aged animals (14-month-old Wistar rats), reported that the impact of exercise on hippocampal function within the rat brain is not markedly affected by age. The age-associated decline in performance of the passive avoidance test, representing both short- and long-term memory, was prevented by exercise. In addition, performance on the pole-jumping active avoidance test, another form of hippocampal-dependent learning, was significantly improved by exercise in middle-aged rats. In contrast, Kim and colleagues (2004) demonstrate a more potent enhancing effect of exercise on cell proliferation in an 8-week-old group of rodents when compared with 4-week-old and 62-week-old animals. This result suggests that the magnitude of an exercise effect is dependent on age. In the current studies, a comparison of young, middle-aged and aged populations suggests that the most robust exercise-induced enhancement in LTP was observed in aged animals. This result may suggest that there is differential effect of age on the potential for exercise to improve LTP.

4.4.2 The effect of exercise on spatial learning

Literature investigating the effect of exercise on spatial learning is inconsistent, especially where research focuses on aged animals. The results from these investigations suggest that an acute forced exercise program does not impact spatial learning, assessed by the MWM, in young or aged animals. Results from middle-aged animals are not discussed since statistical analysis was not valid due to a small exercising group. There are a number of studies that show improvements in spatial learning following exercise in aged rodents; both

voluntary and forced exercise programs have been used. Van Praag and colleagues (2005) demonstrated an improvement in spatial learning in aged mice that had engaged in 45 days of voluntary wheel running. Nichol and colleagues (2007) reported that 3 weeks of voluntary wheel exposure improves cognitive performance, assessed by the radial arm maze, in the aged mouse. Albeck and colleagues (2006) showed an enhancement in spatial learning in aged rats following 7 weeks of mild forced treadmill exercise. In contrast, there is also research that does not support an exercise-effect on spatial learning. Barnes and colleagues (1991) did not observe an enhancement in spatial learning in aged rats following a 10-week exercise program using a motorised treadmill. Additionally, Hansalik and colleagues (2006) investigated the effects of voluntary and forced exercise on the age-related impairment in MWM behaviour, and did not report any positive effect of exercise on this from of learning.

It is clear that exercise may, under certain circumstances, enhance spatial learning in aged rodents. The inconsistency in the literature may be explained by the variability in study design. It is likely that there are differential effects of short- and long-term exercise programs. This theory is supported by Molteni and colleagues (2002) who identified a differential effect of acute and chronic exercise on plasticity-related hippocampal genes. Additionally, Kim and colleagues (2003) highlighted the significance of the intensity and duration of exercise in modulating cell proliferation in the dentate gyrus of rats. It is speculated that an extended exercise period is necessary to consistently show enhancements in spatial learning in aged rodents.

4.4.3 How are the effects of exercise on hippocampal function mediated?

BDNF has frequently been proposed as a key player in mediating the effects of exercise on cognitive function (Berchtold *et al.*, 2005; Chen *et al.*, 2005; Vaynman *et al.*, 2004b). An exercise-induced increase in the concentration of BDNF in the dentate gyrus of young animals was observed, this result has been discussed. There was no exercise-induced effect on the concentration of BDNF in middle-aged or aged animals. Interestingly, previous studies drawing a link between BDNF and exercise-induced effects on brain function have focused on young animals exercising for short periods (Chen *et al.*, 2005; Neeper *et al.*, 1996;

Vaynman *et al.*, 2003). It is possible that an alteration in the induction of BDNF in aged animals explains why no exercise-induced effect on BDNF was observed in the current studies. Indeed, Adlard and colleagues (2005) investigated the effect of age on the induction of BDNF protein in the hippocampus following 4 weeks voluntary exercise. Young, (2-month-old), late middle-aged (15-month-old) and old (24-month-old) mice demonstrated a biphasic profile of BDNF protein induction. Significant increases in BDNF were seen after 1 week of exercise and were followed by a decrease at 2 weeks to values observed in tissue prepared from sedentary rats. Beyond this, BDNF protein levels were only increased significantly, compared to baseline, in young animals. These results suggest that an exercise-induced expression of BDNF within the hippocampus varies across lifespan. Despite this, it is equally possible that a different mechanism subserves an exercise-induced improvement in hippocampal function in older rats.

There is limited research that has explored the role of the neurotrophin receptor sub-types in mediating the effects of exercise on cognitive function. Although there is evidence that implicates changes in BDNF and associated changes in TrkB to improvements in cognitive function in young animals, there is minimal research assessing exercise-induced changes in p75. Given that many studies have emphasised a link between BDNF and exercise-induced effects on cognitive function, it is a logical hypothesis that exercise-induced changes in its receptor TrkB, are also involved in enhancing hippocampal function. There was no exercise-induced effect for either neurotrophin receptor sub-type in young animals; these results have been discussed. Similarly, there was no effect of exercise on either receptor in middle-aged animals. However, there was no marked effect of exercise on hippocampal function in middle-aged animals and it is unlikely that cellular changes associated with cognitive enhancement would be observed. Interestingly, there was an exercise-induced increase in the expression of TrkB and a concomitant reduction in p75 in the dentate gyrus of aged animals. This is particularly interesting as it provides evidence that different mechanisms may underlie the exercise-induced effects on hippocampal function in young and aged animals. Considering previous research, both Widenfalk and colleagues (1999) and Klintsova and colleagues (2004) demonstrate exercise-induced changes in TrkB expression. These studies have already been discussed in detail.

For the most part, it has been proposed that Trk receptors and p75 receptors have antagonistic effects on the fate of the cell. Where Trk receptor activation promotes cell signalling that effectively leads to cell survival, differentiation, neurite outgrowth and activity dependent plasticity (Dan., 2004) p75 activation is generally associated with signalling that negatively impacts the cell (Kohn *et al.*, 1999). Thus, it seems plausible that an increase in the expression of TrkB in response to exercise may be paralleled by a reduction in the expression of p75. Indeed, a decrease in the expression of p75 in the dentate gyrus of aged rats was observed in these studies. This result warrants further investigation since there is very little research investigating whether changes in p75 underlie exercise-induced changes in hippocampal function. The only other study that appears to have investigated the possibility that there is an exercise effect on p75 reported that there were no changes in p75 expression in the sensorimotor cortex, or the hippocampus, following exercise in SHR (Widenfalk *et al.*, 1999).

There is a large body of evidence that suggests a crucial role of MAPK signalling in neuronal functions associated with synaptic plasticity and hippocampal-dependent behaviour (Mazzucchelli & Brambilla, 2000; Orban *et al.*, 1999; Walz *et al.*, 1999). There is also research that specifically implicates ERK activation, which has been associated with an up-regulation of BDNF, in mediating the effects of exercise on cognitive function (Shen *et al.*, 2001; Ying *et al.*, 2002). No changes in ERK activation were observed following an acute forced exercise program in young, middle-aged or aged rats. Given that an increase in ERK activation has been associated with an exercise-induced increase in BDNF, it is more likely that an increase in ERK activation would be observed in young animals, as an exercise-induced increase in BDNF was limited to this population. The results for young animals have been discussed previously. On the basis of results produced by the current investigations, it is proposed that different cellular mechanisms may mediate exercise-induced changes in an aged population and may explain why changes in ERK activation were not observed. In addition, it is also noteworthy that other growth factors and other signalling cascades, for example the PI-3K/Akt pathway (Chen & Russo-Neustadt, 2005), are involved in mediating the effects of exercise on cognitive function. With particular relevance to an aged population it is also possible that anti-inflammatory effects are

contributing to exercise-induced effects on learning and memory. Indeed, it has been speculated that a common mechanism underlying central and peripheral effects of exercise may be related to inflammation, which can impair growth factor signalling both systemically and in the brain (Cotman *et al.*, 2007).

4.4.4 The effect of age on LTP

Considering age-related changes in hippocampal function it is surprising that, although there was a trend suggesting an impairment of LTP with age, there were no significant differences between age groups. Kempermann and colleagues (2002) reported that neurogenesis declines precipitously with age and this impairment in neural plasticity may underlie the age-related loss of hippocampal integrity. The hippocampus is especially vulnerable to the aging process (Burke & Barnes, 2006) and it has been frequently reported that LTP is impaired with age (Eckles-Smith *et al.*, 2000; McGahon *et al.*, 1999; O'Donnell *et al.*, 2000). Where no marked impairment in LTP was observed in the current studies it is possible that the LTP response may, to some extent, be animal specific. Maher and colleagues (2005) reported that a subgroup of aged animals failed to sustain LTP in perforant path granule cell synapses following tetanic stimulation. This highlights that, specifically within aged populations, significant variability in the expression of LTP may exist. Given that there were a maximum of 6 animals per group for aged populations it is possible that this inherent variability to some extent masked a significant decline in LTP with age. It is speculated that a larger sample size would have more clearly shown an age-related impairment of LTP. Interestingly, on closer analysis of the data, it was revealed that a smaller number of control animals in aged populations, when compared with young, demonstrated an increase in EPSP post HFS (it is generally accepted that potentiation following HFS is evidenced by % EPSP values greater than 110 % of baseline immediately post HFS). In young control animals 2 rats (n=6) did not show an increase in EPSP where in aged animals 3 rats (n=6) did not show an increase in EPSP post HFS. Due to small group sizes these observations could not be analysed statistically. Alternatively, if test shocks were delivered for a longer period of time post HFS it is possible that an age-related impairment in the ability to sustain LTP may have been observed.

4.4.5 The effect of age on spatial learning

Analysis of data by 2-way ANOVA did not reveal an effect of age on MWM performance but did reveal an effect of day in young animals. The latter result suggests that only young animals learnt the task. In addition, analysis by 1-way ANOVA of escape latency data from all age groups on separate days, revealed that young animals were faster at finding the submerged platform than aged groups on day 2, 3 and 5 of the MWM task. Collectively these results suggest that there was an age-related decline in spatial learning. This result is consistent with other research that shows a natural decline in spatial learning with age (Fordyce & Wehner, 1993; Geinesman *et al.*, 2004). It is possible that a faster swim speed may account for the reduced escape latency observed in young animals. Analysis of the swim speed and pathlength in young, middle-aged and aged animals revealed that young animals were significantly faster swimmers and swam for significantly shorter distances than aged groups. It is not surprising that young animals swam faster than aged animals. Nevertheless, because a faster swim speed may translate to a faster escape latency, it may be argued that young animals did not necessarily learn better than age-matched rodents but performed better in the task because they swam faster. However, young animals also had a significantly shorter pathlength than aged animals and similar to escape latency, pathlength data reflects performance in the task. A shorter pathlength indicates that the animal has learnt the task. Thus, regardless of swim speed, the results for escape latency and pathlength provide clear evidence of a spatial learning impairment with age.

4.4.6 What cellular changes underlie age-related impairments in hippocampal function?

There was no effect of age on the concentration of BDNF in the dentate gyrus. It has been suggested that neurogenesis declines precipitously with age (Kempermann *et al.*, 2002). It is also known that BDNF has a stimulatory effect on neuronal differentiation (Cheng *et al.*, 2003). The relevance of this is that neurogenesis has been tightly correlated with improved cognitive function, and it may be hypothesised that changes in BDNF underlie learning and memory impairments observed during senescence. Indeed, Hattiangady and colleagues

(2005) produced evidence showing that BDNF concentration declines by middle age (12 months) in the dentate gyrus and the CA1 and CA3 subfields of the rat hippocampus. This group also report that no significant decreases in BDNF were observed between middle-age and old age. Additionally, Gooney and colleagues (2004) suggest that an age-related impairment in LTP may be derived from impairments in BDNF signalling. Given that no significant age-related decline in LTP was seen in these experiments it is possible that the age-related impairment in spatial learning observed is more closely associated with alternative biochemical alterations.

Interestingly, an age-related decrease in the expression of proBDNF in the dentate gyrus was demonstrated. This is a surprising result as proBDNF has been cited as an apoptotic ligand and one might expect proBDNF to be increased with age. However, it is important to appreciate that the literature on pro-neurotrophins is in its infancy. It is also important to acknowledge the complexity of the pro-neurotrophins and their actions. It has been suggested that these compounds induce a number of signalling pathways and may lead to apoptosis or cell survival depending on the particular cytoplasmic factor that has been recruited and the cellular context (Dan, 2004). Thus, it is conceivable that pro-neurotrophins, by activation of the p75 receptor, do not always induce cell death and may, in some circumstances, produce actions that promote cell survival. In addition, it is important to highlight that, in the current studies, the expression of proBDNF in homogenate was analysed, which does not allow identification of its cellular localisation of expression. In relation to the actions of neurotrophins and their proforms, the complexity of the multiple ligand, multiple receptor signalling system is further complicated by the discovery that pro-neurotrophins may be cleaved both intracellularly and extracellularly to yield the mature ligands. Thus, the source of proBDNF is of particular relevance to its biological action and ultimately the fate of the cell. Assessment of age-related changes in secretion of proBDNF would provide additional insights into its role in age-related neurodegenerative processes.

There was no age-related effect on ERK activation in the dentate gyrus. Zhen and colleagues (1999) demonstrated that ERK signalling pathways are impaired in the aged brain. The results revealed an age-associative selective impairment in

MAPK signalling pathways in the rat cortex. Williams and colleagues (2007) also reported that an age-dependent loss of NGF signalling in the rat basal forebrain is due to disrupted MAPK activation. For the current experiments, ERK activation was analysed in the dentate gyrus. Although one might presume that age-related changes in ERK activation would also be evident in this brain region, this has not yet been clearly established. Moreover, since there were no age-related changes in BDNF, TrkB or p75 observed here, it is not surprising that ERK activation was not affected by age.

Many factors have been associated with an age-related decline in cognitive function. The biochemical analysis completed in these investigations was focused specifically on BDNF and associated signalling molecules, which may explain why very few cellular changes were observed. Sonntag and colleagues (2005) reported that growth hormone and IGF-1 replacement to aged animals increases neurogenesis, vascular density and glucose utilizaton, and alters NMDA receptor subunit composition in brain areas that are implicated in learning and memory. Although not expressly linked to changes in cognitive function, these results provide valuable insight into the influence of these hormones on neuronal events in aged animals. Equally important, is the wealth of research that has focused on inflammation in the aging brain. Griffin and colleagues (2006) report that the age-related attenuation in LTP is associated with microglial activation. Inflammatory changes are repeatedly shown to accompany the aging process and may negatively impact brain function, in severe cases causing neurodegeneration. Other factors, such as a decline in neurogenesis (Kempermann *et al.*, 2002) are also likely to play a role. Furthermore, age-related changes in the secretion patterns of the hypothalamic-pituitary adrenal axis have been attributed to an age-related decline in hippocampal function (Miller & O'Callaghan, 2005).

It is clear that many mechanisms and cellular modifications are associated with an age-related impairment in learning and memory. Although not within the scope of the present studies, investigations exploring the contribution of other mechanisms to an age-related impairment in cognitive function would provide valuable information that may elicit a better understanding of how aging impacts hippocampal function. It is proposed that the cellular changes attributable to an age-related impairment in cognitive function are highly complex and have

specific functional roles in relation to hippocampal function.

4.4.7 Summary

The results of this study provide evidence that acute forced exercise can enhance hippocampal synaptic plasticity, specifically LTP, within aged populations. Spatial learning was unaffected by exercise in young, middle-aged and aged populations. Exercise-induced changes in hippocampal function were associated with an increase in the concentration of BDNF in young animals. In aged animals, exercise-induced changes were associated with elevated TrkB and reduced p75 in the dentate gyrus. It is proposed that 1-week of forced exercise has the potential to produce selective enhancements in hippocampal function of young and aged animals. In addition, it is speculated that the mechanisms mediating these effects are dependent on age-status.

There was no obvious impairment of LTP with age but a significant age-related impairment in spatial learning was observed. The age-related impairment in hippocampal function was associated with a decrease in the expression of proBDNF in the dentate gyrus. It is proposed that there is interplay amongst the many cellular mechanisms underlying age-related impairments in hippocampal function, and that these mechanisms may be specific to different forms of hippocampal function.

Chapter 5

An investigation into the effect of chronic exercise on LTP and spatial learning in differently aged populations of rats. What cellular changes underlie cognitive enhancement following chronic exercise?

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An investigation into the effect of chronic exercise on LTP and spatial learning in differently aged populations of rats. What cellular changes underlie cognitive enhancement following chronic exercise?

5.1 Introduction

It is well-recognized that the normal aging process negatively impacts hippocampal function and that cognitive impairment is a natural consequence of age (Burke & Barnes, 2006). Symptoms of hippocampal dysfunction and cognitive alteration are observed with severe cognitive decline such as with Alzheimer's disease and other forms of dementia (Small *et al.*, 1999). Demographic trends have shown that the elderly population is expanding and it may be presumed that the prevalence of neurodegenerative disorders will increase as this population continues to grow. Thus, it is hugely promising that both basic animal models and clinical data overwhelmingly support the theory that exercise intervention is a major protective factor against neurodegeneration of varied etiologies (Kiraly & Kiraly, 2005). Having established that exercise has the potential to confer neuroprotection it is important to clarify whether different exercise programs produce differential effects on cognitive function. More specifically, do acute and chronic exercise regimes similarly impact cognitive function?

The study designs used to investigate the effects of exercise on brain function are widely diverse. A variety of exercise programs, animal populations, animal species and cognitive assessments have been used to determine the effects of exercise on cognitive function. Despite this diversity there is strong evidence that exercise has a positive effect on the brain. It is especially encouraging that exercise has the ability to enhance learning and memory in aged animals. Interestingly, there is little research that has directly focused on exploring the extent to which the variations in study design may affect exercise-induced changes within the brain. Kim and colleagues (2003) reported that cell proliferation in the dentate gyrus is modulated by the intensity and duration of exercise. Molteni and colleagues (2002) found a differential effect of acute and chronic exercise on plasticity related genes in the hippocampus. These results

clearly highlight that different exercise programs can have different effects on the brain. Further research is warranted to more clearly define the effects of acute and chronic exercise on hippocampal function. The ultimate goal will be to determine the optimal exercise conditions for enhancing cognitive function in an aged population.

The molecular mechanisms underlying the exercise-induced enhancements in brain function remain unclear and the extreme variability in study design makes it increasingly difficult to find consistent agreement within the literature. It has been repeatedly suggested that BDNF is a key player in mediating the effects of exercise on cognitive function and a number of signalling cascades associated with BDNF have also been implicated in these processes. However, it is becoming ever more clear that other factors may play contributory roles. In addition, it is possible that the mechanisms involved are age-dependent, time-dependent and possibly specific to the type of exercise or form of hippocampal function.

The aim of this study was to identify the effect of chronic exercise on LTP and spatial learning in differently aged populations of rats and to explore the underlying cellular mechanisms involved. A comparison of the effect of acute and chronic exercise programs on cognitive function will be completed. Finally, an assessment of age-related changes in LTP, spatial learning and associated cellular modifications will be completed.

5.2 Materials and Methods

24 young rats and 28 middle-aged rats were used for these experiments; on completion of the exercise program animals were age-matched to middle-aged and aged groups used in previous studies. Animals were weighed at the beginning of the experimental period, at 4 months and at 8 months (when the exercising period was complete), to give an indication of general health.

Following familiarization to the motorized treadmills, animals were equally divided into control and exercising groups. The chronic exercise program consisted of 3 days of exercise per week for a period of 8 months. Exercising days were always separated by at least one rest day. During the first week of exercise the speed and duration of exercise sessions was gradually increased from 2 x

30min sessions at a speed of 15m/min, to 2 x 40min sessions at a speed of 17m/min. Following this animals ran for 2 x 40min sessions at a speed of 17m/min for the duration of the 8 months. Inter-session rest periods were 30min. The duration and intensity of exercise sessions was similar to that used for acute exercise studies and ensured that animals ran for at least 1km per exercising day. On occasion, the older animals were less keen to run. In these situations a combination of (a) increasing the intensity and or frequency of the current passing through the wire loops and (b) prodding were used to encourage them to exercise; this was generally effective. The animals did not display any signs of undue stress in response to this treatment. Control rats were placed on stationary treadmills for the same duration as age-matched exercising animals. In the week following completion of the exercise program animals were assessed for LTP or spatial learning. At the end of the exercise program a group of young control animals (n=16) were assessed for LTP and spatial learning for comparison with middle-aged and aged groups. Young animals were habituated to their environment and handled for 2-3 days prior to LTP and MWM experiments.

12 middle-aged animals (control n=6, exercised n=6) and 14 aged animals (control n=7, exercised n=7) were used for LTP experiments. Within the aged group 2 animals died (both control rats) during the course of the experiments. The procedure used for the assessment of LTP was identical to that used in all other LTP studies (section 2.5). 12 middle-aged and 12 aged animals (control n=6, exercised n=6 for both populations) were used for the MWM experiments. Within the middle-aged group, 1 control animal died during the course of the experiments. The probe test was completed at least 2hr after the MWM training on day 5, otherwise, the MWM task was identical to that used in other studies (section 2.7).

Rats were sacrificed immediately after completion of the LTP and MWM experiments. First, the weight of the brain, hippocampus, heart and liver were recorded. Following this, the dentate gyrus was dissected (tissue was sliced or homogenised in Krebs solution) and stored as described previously (section 2.8). Some of the dentate gyrus was stored in RNA later at 4°C for analysis of mRNA.

Tissue homogenate (dentate gyrus) was thawed and spun at 14,000 rpm for 5min to extract the supernatant for analysis of BDNF concentration (section 2.10 and

2.10.1) and IL-1β concentration (section 2.10.2) by ELISA. Dentate gyrus samples were analysed for the expression of proBDNF, the neurotrophin receptor subtypes (TrkB and p75) and ERK by gel electrophoresis and western immunoblotting (sections 2.11, 2.12, 2.13 and 2.14). Levels of mRNA in the dentate gyrus were also analysed using RT-PCR (section 2.15). A number of different target genes were quantified by RT-PCR to provide a better understanding of the interplay of mechanisms involved in mediating the exercise-induced effects on the brain; these are listed in Table 2.1. Data are presented as relative quotient (RQ) values, which represent a fold difference relative to the average for young control animals.

Correlation analysis was completed to identify whether there was a relationship between hippocampal function, assessed by LTP or spatial learning, and any of the measured cellular parameters.

When identifying cellular changes results from LTP and MWM experiments were analysed individually. Where no effect was observed age-matched data were pooled from control animals, and separately from exercised animals, to identify if an exercise effect would be revealed with a larger sample size. Results showing changes in protein and gene expression are presented as pooled data unless otherwise specified.

5.3 Results

5.3.1 Body Weight

Body weight measurements were taken at the start of the exercise period, at 4 months and at 8 months for control and exercising animals. Body weight was significantly increased in all groups during the 8 months (Figure 5.1a, b, c & d). The results for middle-aged control animals were 364.7 ± 9.4, 437.5 ±12.1 and 476.8 ±12.8 g at the start of experiments, 4 months and 8 months respectively. Results for middle-aged exercised animals were 384.3 ± 7.4, 453.9 ± 9.4 and 483.5 ± 10.4 g at the start of experiments, 4 months and 8 months respectively. The results for aged control animals were 448.5 ± 7.8, 478.1 ± 8.4 and 497.2 ± 10.4 g at the start of experiments, 4 months and 8 months respectively. Results for aged exercised animals were 424.4 ± 6.8, 432.3 ± 6.5 and 450.82 ± 7.1 g at the start of experiments, 4 months and 8 months respectively.

Aged exercising animals had a significantly reduced body weight compared with age-matched controls at the start of experiments ($p<0.05$), at 4 months ($p<0.001$) and at 8 months ($p<0.001$). At the start of experiments results were 448.5 ± 7.8 and 424.2 ±6.8 g for control and exercising animals respectively. At 4 months results were 478.1 ± 8.4 and 432.3 ± 6.5 g for control and exercising animals respectively. At 8 months results were 497.2 ± 10.4 and 450.8 ± 7.1 g for control and exercising animals respectively.

5.3.1.1 Organ weights

Organs were weighed immediately following sacrifice of the animal. Results for brain, hippocampus, heart and liver weight for middle-aged and aged animals are given in Table 5.1 (* symbol indicates a significant difference between middle-aged and aged control animals).

WEIGHT (g)	CONTROL	EXERCISED
BRAIN Middle-Aged	2.02 ± 0.08	2.13 ± 0.05
BRAIN Aged	2.10 ± 0.02	2.07 ± 0.03
HIPPOCAMPUS Middle-Aged	0.17 ± 0.01	0.15 ± 0.01
HIPPOCAMPUS Aged	0.15 ± 0.003	0.15 ± 0.004
HEART Middle-Aged	1.37 ± 0.05	1.43 ± 0.05
HEART Aged	* 1.13 ± 0.10	1.14 ± 0.03
LIVER Middle-Aged	13.52 ± 0.92	12.46 ± 0.50
LIVER Aged	* 12.04 ± 0.39	11.43 ± 0.21

Table 5.1 Weight measurements for brain, hippocampus, heart and liver of middle-aged and aged animals

There was no effect of exercise on hippocampal weight (expressed as a percentage of brain weight), heart weight or liver weight. Hippocampal weight (expressed as a percentage of brain weight), was significantly decreased with age ($p<0.01$). Results were 8.8 ± 0.62 and 7.0 ± 0.17 % brain weight for middle-aged and aged animals respectively (Figure 5.3a). Weight measurements for the heart and liver were also reduced significantly with age (Figure 5.3b & c). Heart weight measurements were 1.37 ± 0.05 and 1.13 ± 0.1 g for middle-aged and aged animals respectively ($p<0.05$). Liver weight measurements were, 13.52 ± 0.92 and 11.43 ± 0.21 g for middle-aged and aged animals respectively ($p<0.05$).

5.3.2 The effect of chronic exercise on LTP

The effect of a chronic 8-month forced exercise program on LTP in middle-aged and aged rats was assessed. Young control animals (n=8) were also assessed for LTP. No significant exercise-effect was observed in middle-aged or aged animals when analysing the data using a 2-way ANOVA. Analysis of the mean % EPSP (± SEM) during the last 5min of the experiment gave an indication of the ability to sustain LTP. Results for young control animals are shown in Figure 5.4a. The mean % EPSP during the last 5min of the experiment was 121.20 ± 10.22 % EPSP. There was no significant exercise-induced effect on LTP in middle-aged or aged animals. Mean % EPSP for middle-aged animals was 108.0 ± 5.88 and 122.4 ± 9.01 % EPSP for control and exercising animals respectively (Figure 5.4c). Mean % EPSP for aged animals was 108.0 ± 5.57 and 123.5 ± 8.96 % EPSP for control and exercising animals respectively (Figure 5.4e).

5.3.2.1 The effect of acute exercise and chronic exercise on LTP

A comparison of the effects of acute and chronic exercise on LTP in middle-aged and aged animals was assessed. Analysis was completed by 2-way ANOVA; this revealed an effect of acute and chronic exercise in aged animals ($p<0.05$). Data are presented in Figure 5.5b. A 1-way ANOVA was also used to assess differences between groups for mean % EPSP during the last 5min of the experiment; these data give an indication of the ability to sustain LTP. Although there was a trend for an exercise-induced enhancement in LTP in middle-aged animals following both acute and chronic exercise, these differences were not significant (Figure 5.5c). Data were 116.5 ± 5.61 and 144.5 ± 14.33 % EPSP for control and exercising animals following acute exercise, and 108.3 ± 6.02 and 122.6 ± 9.01 % EPSP for control and exercising animals following chronic exercise. For aged animals there was an exercise-induced enhancement in LTP following acute exercise ($p<0.01$). Again, although there was a trend suggesting an exercise-induced enhancement in LTP following chronic exercise this was not found to be significant. Results for aged animals are shown in Figure 5.5d; mean data (± SEM) were 108.6 ± 5.5 and 142.1 ± 3.86 % EPSP for control and exercising animals following acute exercise. Mean data (± SEM) were 107.6 ± 5.65 and 123.4 ± 8.85 % EPSP for control and exercising animals following

chronic exercise. Analysis also revealed a significant difference ($p<0.05$) between aged animals that had completed the acute exercise program and aged animals that had completed the chronic exercise program.

5.3.2.2 The effect of age on LTP

Analysis of data from control animals revealed no effect of age on LTP. A 2-way ANOVA was completed on mean values for % EPSP at all time points post tetanus during the experiment for young, middle-aged and aged rats; no statistical changes were revealed (Figure 5.6a). A 1-way ANOVA was completed to compare values for % EPSP during the last 5min of the experiment (Figure 5.6b). There was no significant difference between young (121.2 ± 10.22 % EPSP), middle-aged (108.0 ± 5.88 % EPSP) and aged (108.0 ± 5.75 % EPSP) control animals that suggests there was no effect of age on LTP. These results are similar to those observed with acute studies.

5.3.3 Effect of chronic exercise on spatial learning

The MWM was used to assess spatial learning in young, middle-aged and aged populations of animals. Results for escape latency for young control animals were 51.8 ± 3.2, 35.58 ± 5.2, 31.95 ± 7.3, 22.32 ± 4.5 and 20.03 ± 3.7 secs for day 1, day 2, day 3, day 4 and day 5 respectively (Figure 5.7a). Analysis by 1-way ANOVA revealed significant differences on day 2 ($p<0.05$), day 3 ($p<0.05$), day 4 ($p<0.01$) and day 5 ($p<0.001$) when compared with day 1. Pathlength was also significantly shorter on day 5 compared with day 1 ($p<0.05$; 831.7 ± 45.1 and 431.7 ± 86. cm for day 1 and day 5 respectively). A 2-way ANOVA was used to determine the effect of chronic exercise or day on escape latency in middle-aged and aged animals. In middle-aged animals analysis by 2-way ANOVA did not reveal an exercise effect but did show significant differences in day of trial in the exercising group. Escape latency for middle-aged control animals was 51.26 ± 1.2, 42.97 ± 5.6, 44.74 ± 5.6, 41.47 ± 6.4 and 33.93 ± 6.8 secs for day 1, day 2, day 3, day 4 and day 5 respectively. Escape latency for middle-aged exercising animals was 52.92 ± 3.1, 45.35 ± 2.7, 38.89 ± 4.9, 27.39 ± 6.7 and 20.81 ± 3.4 secs for day 1, day 2, day 3, day 4 and day 5 respectively. For middle-aged exercising animals analysis by 1-way ANOVA revealed a significant decrease in

escape latency on day 4 ($p<0.01$) and day 5 ($p<0.001$) when comparing performance with that on day 1 (Figure 5.7b). Exercising animals also had a significantly shorter pathlength ($p<0.001$) on day 4 and day 5 when compared with day 1 (Table 5.2). There was no effect of exercise on swim speed in middle-aged animals. Collectively these results show that the exercise group learnt the task but the control group did not, which suggests that there was an exercise-induced effect on spatial learning in middle-aged rats. For aged animals, data analysis by 2-way ANOVA revealed that there was no effect of chronic exercise but that there was a significant effect of day. The latter result suggests that learning occurred in both control and exercising groups (Figure 5.7c). Escape latency for control animals was 47.75 ± 5.7, 27.3 ± 5.0, 25.38 ± 7.6, 25.37 ± 3.4 and 16.67 ± 3.4 secs for day 1, day 2, day 3, day 4 and day 5 respectively. Escape latency for exercising animals was 47.17 ± 4.5, 39.41 ± 2.8, 29.32 ± 6.4, 17.59 ± 2.5 and 22.26 ± 4.4 secs for day 1, day 2, day 3, day 4 and day 5 respectively. On day 2 of the task exercising animals had a significantly greater pathlength ($p<0.05$) than control animals (Table 5.3). There was no effect of exercise on swim speed.

MIDDLE-AGED ANIMALS		SWIM SPEED (CM/SECS)	PATHLENGTH (CM)
DAY 1	Control	21.5 ± 0.7	1080.1 ± 37.6
	Exercised	21.0 ± 1.4	1028.3 ± 27.3
DAY 2	Control	24.9 ± 1.3	970.0 ± 106.7
	Exercised	22.9 ± 0.8	984.7 ± 43.9
DAY 3	Control	22.9 ± 1.4	984.8 ± 110.1
	Exercised	23.7 ± 1.2	888.6 ± 66.2
DAY 4	Control	25.4 ± 2.5	925.0 ± 105.4
	Exercised	25.6 ± 1.9	632.2 ± 84.2
DAY 5	Control	20.0 ± 1.2	741.6 ± 132.8
	Exercised	27.4 ± 2.2	486.1 ± 64.6

Table 5.2 Swim speed and pathlength results for middle-aged animals during the MWM task

AGED ANIMALS		SWIM SPEED (CM/SECS)	PATHLENGTH (CM)
DAY 1	Control	29.0 ± 1.7	1357.0 ± 139.9
	Exercised	26.9 ± 1.2	1284.8 ± 113.3
DAY 2	Control	28.2 ± 0.8	751.1 ± 135.7
	Exercised	29.6 ± 1.6	1164.4 ± 119.8
DAY 3	Control	21.7 ± 1.6	541.0 ± 152.7
	Exercised	24.1 ± 1.5	697.9 ± 149.7
DAY 4	Control	22.6 ± 1.5	557.3 ± 94.6
	Exercised	22.9 ± 1.5	410.3 ± 80.4
DAY 5	Control	21.1 ± 1.5	351.4 ± 96.6
	Exercised	22.9 ± 1.9	529.8 ± 127.9

Table 5.3 Swim speed and pathlength results for aged animals during the MWM task

5.3.3.1 Effect of chronic exercise on probe test performance

For middle-aged animals there was a trend for exercising animals to swim for more time in the NW quadrant during the probe trial compared with age-matched controls (p=0.052). Time swimming in the NW quadrant was 16.56 ± 2.1 and 22.5 ± 1.7 secs for control and exercising groups respectively (Figure 5.8a). This would suggest that the exercised animals learnt the task and support the results for escape latency. Additionally, middle-aged exercising animals had a significant preference for the NW quadrant when analysis of % time in each quadrant was completed. Results were 19.67 ± 2.1, 29.33 ± 2.7, 37.5 ± 2.8 and 13.5 ± 1.8 % for

SW, NE, NW and SE respectively.

For aged animals time swimming in the NW quadrant during the probe test was 28.5 ± 4.1 and 21.7 ± 3.1 secs for control and exercising animals respectively (Figure 5.8b). Interestingly, both groups had a significant preference for the NW quadrant when % time swimming in all quadrants was analysed. Control animals showed a greater preference for the NW quadrant than exercised animals. Data for control animals were 17.45 ± 0.8, 21.22 ± 5.1, 47.5 ± 6.9 and 13.83 ± 3.3 % for SW, NE, NW and SE respectively. Data for exercising animals were 19.05 ± 2.4, 27.43 ± 3.7, 36.10 ± 5.2 and 17.43 ± 3.6 % for SW, NE, NW and SE respectively.

5.3.3.2 Effect of age on spatial learning

Analysis of escape latency data for young, middle-aged and aged control animals was completed to determine if there was an effect of age on spatial learning. Statistical analysis using a 2-way ANOVA revealed a significant effect of age and day of trial on escape latency (Figure 5.9a). On day 4 escape latency was 22.32 ± 4.5, 41.47 ± 6.4 and 25.4 ± 3.4 secs for young, middle-aged and aged animals respectively ($p<0.05$ for young compared with middle-aged and $p<0.05$ for aged compared with middle-aged groups). This result suggests an impairment in spatial learning in middle-aged, but not aged animals. Surprisingly, the results for young and aged animals were similar. To investigate this further, MWM performance of aged control rats used in acute studies and aged control rats used in chronic studies was compared to identify if these groups similarly represented naïve animals. Interestingly, significant differences were revealed between these groups (Figure 5.9b). Significant differences on day 2 ($p<0.01$), day 3 ($p<0.01$), day 4 ($p<0.05$) and day 5 ($p<0.01$) were observed. Escape latency results for control animals on day 2 were 52.33 ± 4.6 and 27.3 ± 5.0 secs, on day 3 were 49.33 ± 4.0 and 25.38 ± 7.6 secs, on day 4 were 44.17 ± 5.8 and 25.37 ± 3.4 secs and on day 5 were 44.0 ± 5.5 and 16.67 ± 3.4 secs for acute studies and chronic studies respectively. Collectively, these data suggest that aged control rats used in chronic studies had significantly better spatial learning than age-matched control rats used in acute studies. This suggests that aged control rats used in chronic studies were not naïve animals, it is proposed that environmental enrichment enhanced spatial learning in this group.

5.3.3.3 Effect of acute exercise and chronic exercise on spatial learning

A comparison of the effects of acute and chronic exercise on spatial learning in middle-aged and aged animals was assessed. Exercising animals from acute and chronic studies were compared with control animals from acute studies. Given there is speculation that aged control rats used in chronic studies were not naïve animals, control rats from acute studies were used for comparison with exercised groups. A 2-way ANOVA was used for statistical analysis (Figure 5.10a). For middle-aged exercising animals that completed the chronic exercise program there was a significant improvement in performance, assessed by escape latency, on day 4 ($p<0.05$) and day 5 ($p<0.01$) compared with day 1. It is important to note that there were only two animals in the exercising group that completed the acute exercise program, which meant that statistical analysis was not viable. On day 1 escape latency was 52 ± 2.0, 57.0 ± 3.0 and 52.9 ± 3.1 secs for control, acute exercise and chronic exercise groups respectively. On day 4 escape latency was 45.0 ± 5.3, 56.0 ± 4.0 and 27.4 ± 6.7 secs for control, acute exercise and chronic exercise groups respectively. On day 5 escape latency was 41.2 ± 5.9, 39.5 ± 5.5 and 20.8 ± 3.4 secs for control, acute exercise and chronic exercise groups respectively. Cumulatively, these data suggest that chronic exercise positively impacts spatial learning in middle-aged animals.

For aged animals there was no improvement in spatial learning with acute exercise. However, there was a significant decrease in escape latency on day 3 ($p<0.05$), day 4 ($p<0.01$) and day 5 ($p<0.05$) in aged exercising animals that completed the chronic exercise program compared with age-matched controls that suggests there was an exercise-induced improvement in spatial learning (Figure 5.10b). On day 1 escape latency was 54.0 ± 2.3, 51.0 ± 3.8 and 47.2 ± 4.5 secs for control, acute exercise and chronic exercise groups respectively. On day 3 escape latency was 49.3 ± 4.0, 45.7 ± 5.8 and 29.3 ± 6.4 secs for control, acute exercise and chronic exercise groups respectively. On day 4 escape latency was 44.2 ± 5.8, 35.7 ± 7.3 and 17.6 ± 2.5 secs for control, acute exercise and chronic exercise groups respectively. On day 5 escape latency was 44.0 ± 5.4, 36.83 ± 8.9 and 22.26 ± 4.4 secs for control, acute exercise and chronic exercise groups respectively. Similar to middle-aged rats, these results suggest that chronic exercise, but not acute exercise, may enhance spatial learning in aged animals.

5.3.4 Effect of chronic exercise and age on BDNF concentration

The impact of chronic exercise on the concentration of BDNF in the dentate gyrus was investigated in middle-aged and aged animals. An exercise-induced increase in BDNF in middle-aged animals was observed ($p<0.05$). BDNF concentration in the dentate gyrus was 0.04 ± 0.01 and 0.06 ± 0.01 ng/mg for control and exercised animals respectively (Figure 5.11a). For aged animals BDNF concentration in the dentate gyrus was 0.11 ± 0.03 and 0.11 ± 0.02 ng/mg for control and exercising animals respectively (Figure 5.11b). There was no exercise-induced effect on the concentration of BDNF in the dentate gyrus of aged rats.

The effect of age on BDNF concentration in the dentate gyrus was also investigated. Analysis of young, middle-aged and aged control rats was completed. There was a significant increase in BDNF concentration in aged compared with middle-aged animals ($p<0.05$). BDNF expression was 0.08 ± 0.01, 0.04 ± 0.01 and 0.11 ± 0.02 ng/mg for young, middle-aged and aged animals respectively (Figure 5.11c).

5.3.5 Effect of chronic exercise and age on IL-1β concentration

The effect of chronic exercise on the concentration of IL-1β in the dentate gyrus was investigated in middle-aged and aged animals. When analysing tissue for IL-1β concentration a number of negative results were produced. In light of this, it is possible that these results may not be reliable and ideally should be repeated. There was no effect of exercise on the concentration of IL-1β in the dentate gyrus of middle-aged or aged animals (5.12a & b). For middle-aged animals IL-1β concentration was 0.18 ± 0.04 and 0.32 ± 0.07 ng/mg for control and exercising animals respectively. For aged animals IL-1β concentration was 0.33 ± 0.1 and 0.30 ± 0.04 ng/mg for control and exercising animals respectively.

The effect of age on IL-1β concentration in young, middle-aged and aged control animals was also assessed. The effect of age on IL-1β concentration in the dentate gyrus revealed an age-related decline ($p<0.05$; 0.58 ± 0.09, 0.18 ± 0.04 and 0.33 ± 0.1 ng/mg for young, middle-aged and aged animals respectively). Results are shown in Figure 5.12c.

5.3.6 Effect of chronic exercise and age on proBDNF expression

The effect of a chronic forced exercise program on the expression of proBDNF in the dentate gyrus of middle-aged and aged animals was assessed. There was no significant effect of chronic exercise on proBDNF expression in middle-aged or aged animals. For middle-aged animals the expression of proBDNF was 0.58 ± 0.09 and 0.45 ± 0.05 proBDNF/β-actin for control and exercising animals respectively (Figure 5.13a). For aged animals proBDNF expression was 0.54 ± 0.09 and 0.50 ± 0.07 proBDNF/β-actin for control and exercising animals respectively (Figure 5.13b).

The effect of age on the expression of proBDNF in the dentate gyrus of young, middle-aged and aged animals was also analysed. Statistical analysis was completed using a 1-way ANOVA. Unlike the observations from acute studies, there was no effect of age on proBDNF concentration. The expression of proBDNF was 0.49 ± 0.08, 0.58 ± 0.09 and 0.54 ± 0.09 proBDNF/β-actin for young, middle-aged and aged animals respectively (Figure 5.13c).

5.3.7 The effect of chronic exercise and age on TrkB expression

The effect of a chronic forced exercise program on the expression of TrkB in the dentate gyrus of middle-aged and aged animals was investigated. There was no effect of chronic exercise on TrkB expression in middle-aged or aged animals. TrkB expression was 4.54 ± 0.65 and 3.23 ± 0.42 TrkB/β-actin for control and exercising middle-aged animals respectively (Figure 5.14a). TrkB expression was 3.54 ± 0.61 and 4.03 ± 0.43 TrkB/β-actin for control and exercising aged animals respectively (Figure 5.14b).

Similar to the acute studies the effect of age on TrkB expression in the dentate gyrus was assessed. Statistical analysis was completed using a 1-way ANOVA. Although there may be a trend for an age-related decrease in TrkB, no significant differences between groups were identified (Figure 5.14c). TrkB expression was 4.49 ± 0.56, 4.54 ± 0.65 and 3.54 ± 0.61 TrkB/β-actin for young, middle-aged and aged animals respectively.

5.3.8 The effect of chronic exercise and age on p75 expression

The effect of acute exercise on p75 expression in the dentate gyrus of middle-aged

and aged animals was investigated. No exercise-induced effects on p75 expression in middle-aged or aged animals were observed. The expression of p75 was 0.14 ± 0.02 and 0.13 ± 0.01 p75/β-actin for control and exercising middle-aged animals respectively (Figure 5.15a). For aged animals, p75 expression was 0.15 ± 0.03 and 0.13 ± 0.02 p75/β-actin for control and exercising animals respectively (Figure 5.15b).

The effect of age on p75 expression in the dentate gyrus of young, middle-aged and aged control animals was also assessed. Statistical analysis was completed using a 1-way ANOVA. No effect of age on the expression of p75 was identified (Figure 5.15c). The concentration of p75 was 0.18 ± 0.02, 0.14 ± 0.02 and 0.15 ± 0.03 p75/β-actin for young, middle-aged and aged control animals respectively.

5.3.9 The effect of chronic exercise and age on ERK activation

The effect of chronic exercise on ERK activation in the dentate gyrus of middle-aged and aged animals was investigated. There was no exercise-induced effect on ERK activation in either age group. ERK activation for middle-aged animals was 0.24 ± 0.06 and 0.28 ± 0.07 p44/tERK (arbitrary units) for control and exercising animals respectively (Figure 5.16a). For aged animals, ERK activation was 0.19 ± 0.04 and 0.21 ± 0.04 p44/tERK (arbitrary units) for control and exercising animals respectively (Figure 5.16b).

The effect of age on ERK activation in the dentate gyrus was assessed by comparing young, middle-aged and aged control animals. Statistical analysis was completed using a 1-way ANOVA. There was no effect of age on ERK activation in the dentate gyrus. ERK activation was 0.23 ± 0.03, 0.24 ± 0.06 and 0.20 ± 0.04 p44/tERK (arbitrary units) for young, middle-aged and aged animals respectively (Figure 5.16c).

5.3.10 Analysis of mRNA in the dentate gyrus; effect of chronic exercise and age

Levels of mRNA in the dentate gyrus were quantified using RT-PCR.

5.3.10.1 Analysis of BDNF mRNA

The RQ of BDNF mRNA in the dentate gyrus of young control, middle-aged

control, middle-aged exercising, aged control and aged exercising animals was calculated. Interestingly, in tissue prepared from middle-aged rats that had completed LTP experiments there appears to be a trend for a decrease in BDNF mRNA with exercise. Additionally, in tissue prepared from middle-aged rats that had completed MWM experiments there appears to be a trend showing an exercise-induced increase in BDNF mRNA. Despite these observations, there was no significant effect of chronic exercise on BDNF mRNA in LTP or MWM experiments. In LTP experiments there was a significant increase in BDNF mRNA in middle-aged control rats compared with young control rats ($p<0.01$). RQ values were 1.21 ± 0.51, 6.43 ± 1.21, 3.13 ± 1.26, 3.66 ± 0.98 and 2.24 ± 0.71 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.17a). Interestingly, in MWM experiments BDNF mRNA was significantly reduced with age ($p<0.001$). RQ values were 1.08 ± 0.08, 0.49 ± 0.05, 1.10 ± 0.33, 0.43 ± 0.10 and 0.62 ± 0.14 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.17b).

Looking specifically at the data for middle-aged control rats in LTP and MWM experiments (Figure 5.17a & b) there appears to be a decrease in mRNA BDNF in MWM experiments compared to the level in LTP experiments. A similar difference is observed in aged animals. The only explanation that may be provided is that there was a significant amount of stress induced by completing the MWM task and this induced a reduction in BDNF mRNA.

5.3.10.2 Analysis of NT3 and NT4/5 mRNA

The RQ of NT3 and NT4/5 mRNA in the dentate gyrus of young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals was calculated. There was no effect of chronic exercise or age on NT3 mRNA (Figure 5.18a). RQ values were 1.18 ± 0.20, 1.06 ± 0.17, 1.1 ± 0.15, 1.0 ± 0.13 and 0.98 ± 0.12 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively. For LTP experiments although there was no significant effect of chronic exercise or age on NT4/5 mRNA there is a trend that suggests an age-related increase (Figure 5.18b). RQ values were 1.37 ± 0.26, 4.23 ± 1.10, 4.95 ± 0.78, 3.8 ± 1.24 and 2.99 ± 0.46 for

young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively. For MWM experiments (Figure 5.18c) there was a significant decrease in NT4/5 in middle-aged exercising animals compared with age-matched controls ($p<0.05$). In addition, there was an increase in NT4/5 in middle-aged control animals compared with young control animals ($p<0.05$). RQ values were 1.12 ± 0.16, 2.05 ± 0.35, 0.80 ± 0.23, 1.61 ± 0.27 and 2.33 ± 0.33 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively.

5.3.10.3 Analysis of TrkA, TrkB and TrkC mRNA

The RQ of TrkA, TrkB and TrkC mRNA in the dentate gyrus of young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals was calculated. There was no effect of chronic exercise or age on mRNA of any of the tyrosine kinase receptors (Figure 5.19a, b & c). RQ values for TrkA were 1.42 ± 0.28, 1.83 ± 0.48, 2.02 ± 0.52, 1.15 ± 0.33 and 1.67 ± 0.44 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively. There may be a trend for an age-related increase in TrkB mRNA; RQ values were 1.03 ± 0.04, 1.23 ± 0.08, 1.28 ± 0.09, 1.24 ± 0.08 and 1.22 ± 0.09 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively. RQ values for TrkC were 1.0 ± 0.04, 1.06 ± 0.02, 0.92 ± 0.06, 1.07 ± 0.03 and 1.02 ± 0.05 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively.

5.3.10.4 Analysis of p75 mRNA

The RQ of p75 mRNA in the dentate gyrus of young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals was calculated. For LTP experiments (Figure 5.20a) there was a significant decrease of p75 mRNA in aged exercising animals compared with age-matched controls ($p<0.05$). In addition there was a significant increase in p75 mRNA with age ($p<0.001$ for young and aged control animals, $p<0.05$ for middle-aged and aged animals). RQ values were 1.08 ± 0.15, 3.22 ± 0.74, 2.74 ± 0.60, 5.28 ± 1.17 and 2.68 ± 0.46 for young control, middle-aged control, middle-aged exercising, aged control and

aged exercising animals respectively. For MWM experiments (Figure 5.20b) there is also evidence of an age-related increase in p75 mRNA ($p<0.05$ for young and aged control animals, $p<0.05$ for middle-aged and aged animals). RQ values were 1.06 ± 0.10, 0.97 ± 0.18, 1.8 ± 0.46, 2.12 ± 0.26 and 2.19 ± 0.28 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively.

5.3.10.5 Analysis of IGF-1 and VEGF mRNA

There was no effect of chronic exercise or age on IGF-1 mRNA in the dentate gyrus. RQ values were 1.06 ± 0.07, 1.19 ± 0.06, 1.2 ± 0.10, 1.13 ± 0.12 and 1.05 ± 0.09 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.21a). For MWM experiments there was no effect of exercise or age on VEGF mRNA. There was an age-related increase in VEGF mRNA in middle-aged compared with young control animals in LTP experiments ($p<0.05$). RQ values were 1.02 ± 0.02, 1.47 ± 0.10, 1.30 ± 0.18, 1.30 ± 0.11 and 0.98 ± 0.08 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.21b).

5.3.10.6 Analysis of Ki-67 mRNA

Ki-67 is a marker of cell proliferation and quantities of Ki-67 give an indication of the level of neurogenesis. In LTP studies there was a progressive decline in Ki-67 mRNA with age ($p<0.01$; young and middle-aged control animals, $p<0.001$; young and aged animals). RQ values were 0.99 ± 0.07, 0.45 ± 0.15, 0.50 ± 0.17, 0.33 ± 0.07 and 0.24 ± 0.05 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.22a). In the MWM studies (Figure 5.22b) there appears to be a trend for an exercise-induced increase in Ki-67 in both middle-aged and aged animals but these differences were not found to be significant. Similar to the LTP studies there was an age-related decline in Ki-67 mRNA ($p<0.05$ for young and aged animals). RQ values were 0.99 ± 0.12, 0.59 ± 0.04, 0.79 ± 0.18, 0.41 ± 0.11 and 0.68 ± 0.13 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively.

5.3.10.7 Analysis of IL-1β, IκB and TNF-α mRNA

There appears to be a trend for an exercise-induced decrease in the inflammatory cytokine IL-1β in aged animals (not significant). There is clear evidence of an age-related increase in IL-1β. Middle-aged animals had significantly more IL-1β mRNA than young control animals ($p<0.01$). There appears to be a similar trend for an age-related increase in IL-1β in aged animals when compared with young (not significant). RQ values were 0.92 ± 0.17, 2.21 ± 0.34, 2.47 ± 0.59, 2.0 ± 0.68 and 1.17 ± 0.28 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.23a). The results for inhibitory κB (IκB), another inflammatory marker, show a progressive increase with age ($p<0.05$; young compared with middle-aged animals, $p<0.01$; young compared with aged animals). RQ values for IκB were 1.0 ± 0.17, 1.12 ± 0.05, 1.12 ± 0.04, 1.13 ± 0.06 and 1.22 ± 0.06 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.23b). Surprisingly, there was an exercise-induced increase in TNF-α in aged animals ($p<0.05$) and an age-related decline when comparing young and aged control animals ($p<0.05$). RQ values were 1.06 ± 0.07, 0.81 ± 0.08, 1.10 ± 0.13, 0.61 ± 0.06 and 0.98 ± 0.10 for young control, middle-aged control, middle-aged exercising, aged control and aged exercising animals respectively (Figure 5.23c).

5.3.11 Correlation analysis of LTP and BDNF concentration in the dentate gyrus

Correlation analysis revealed a positive correlation between % EPSP during the final 5min of LTP experiments and BDNF protein concentration in the dentate gyrus ($p=0.0157$, $r^2 = 0.21$). Statistical analysis was completed by linear regression with a 95% confidence interval. Results are shown in Figure 5.24. This result suggests that BDNF concentration is associated with expression of LTP.

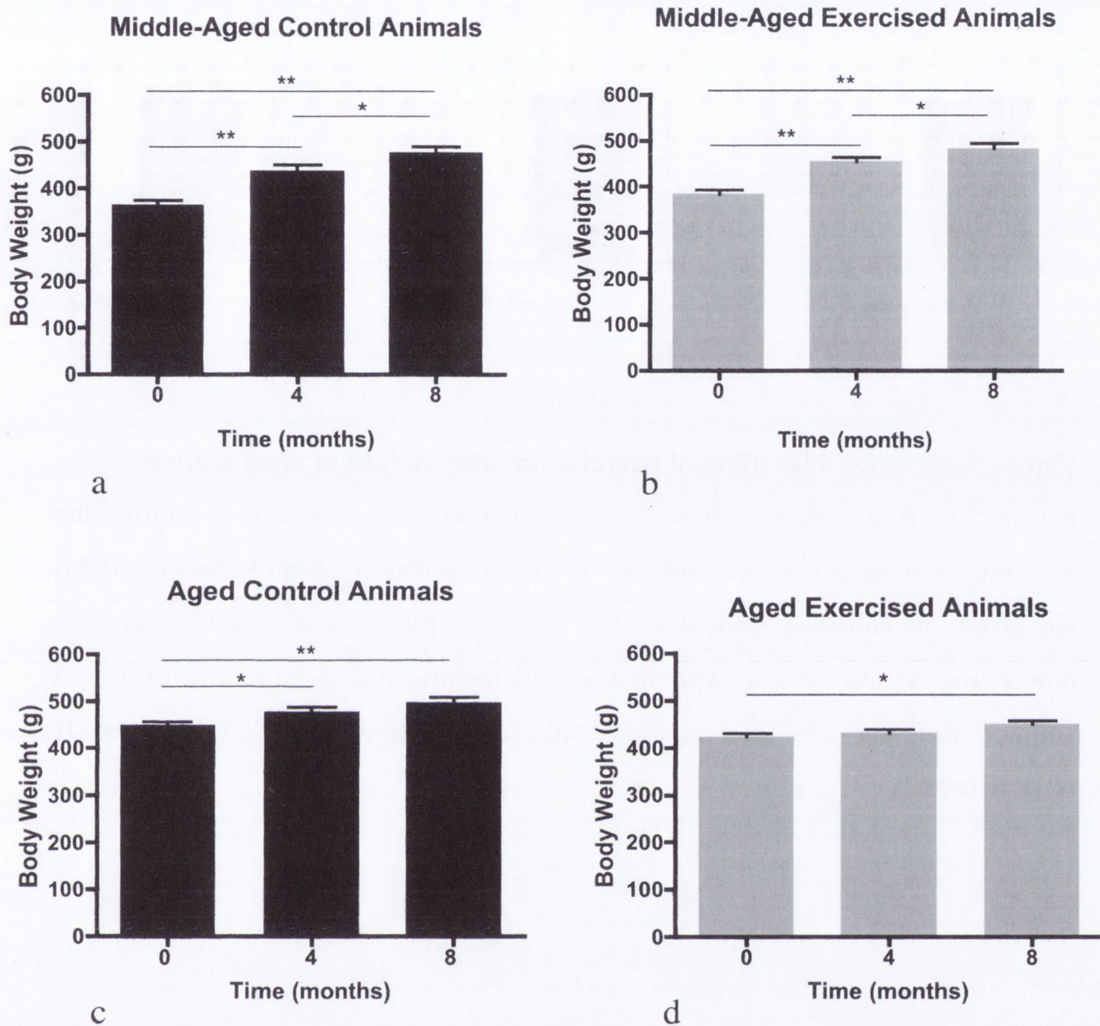


Figure 5.1a, b, c & d Body weight measurements during the 8-month exercise period

The results for body weight measurements of all animals are shown in Figure 5.1a, b, c & d. Data for middle-aged control rats (n=11), middle-aged exercised rats (n=12), aged control rats (n=11) and aged exercised rats (n=13) are presented. Mean values (± SEM) are shown, a 1-way ANOVA was used to assess the effect of age on body weight. There was a significant increase in body weight with age for all groups during the 8-month experimental period.

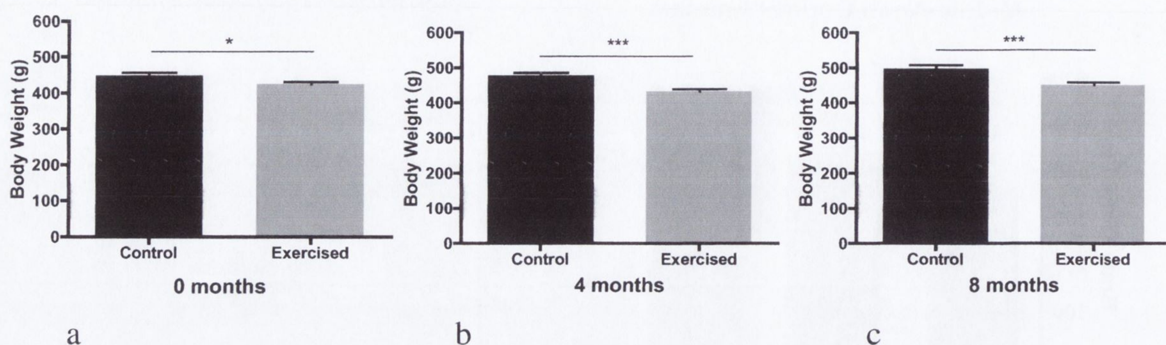


Figure 5.2a, b & c The effect of exercise on body weight in aged animals

Figure 5.2a, b & c show body weight measurements for aged animals during the 8-month exercise period (control n= 11, exercised n=13). Mean values (± SEM) are given, an unpaired Student's t-test was used for statistical analysis at each time point. At the start of experiments, at 4 months and at 8 months, exercised animals weighed less than age-matched controls ($p<0.05$, $p<0.001$, $p<0.001$ respectively).

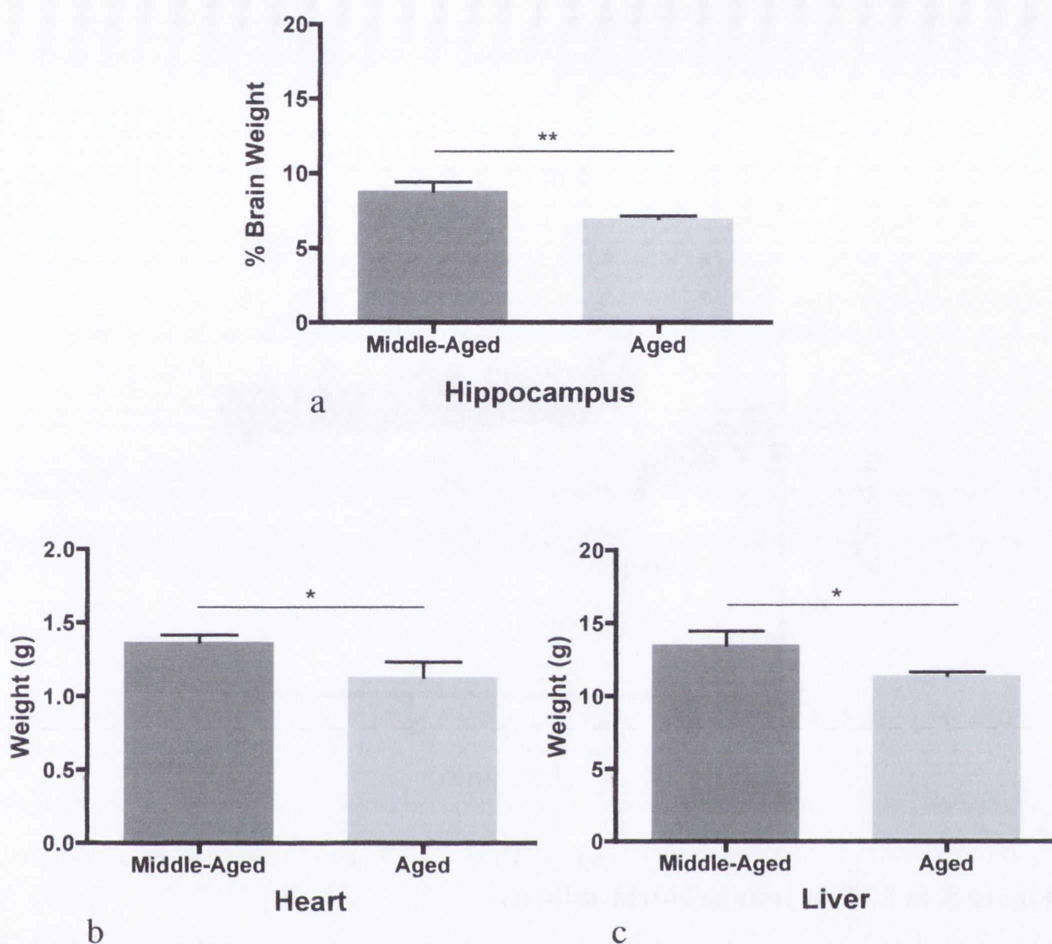
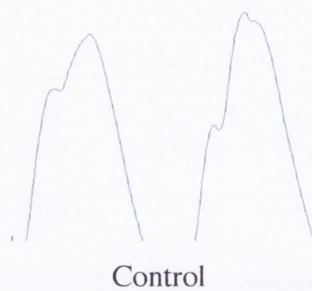


Figure 5.3a, b & c The effect of age on hippocampal weight, heart weight and liver weight

Figure 5.3a, b & c show the results hippocampal weight (expressed as a percentage of brain weight), heart weight and liver weight in control animals. Data for middle-aged control rats (n=11) and aged control rats (n=11) are presented. Mean values (± SEM) are shown, an unpaired Student's t-test was used for statistical analysis of different age groups. There was an age-related decrease in hippocampal weight ($p<0.01$), heart weight ($p<0.05$) and liver weight ($p<0.05$).



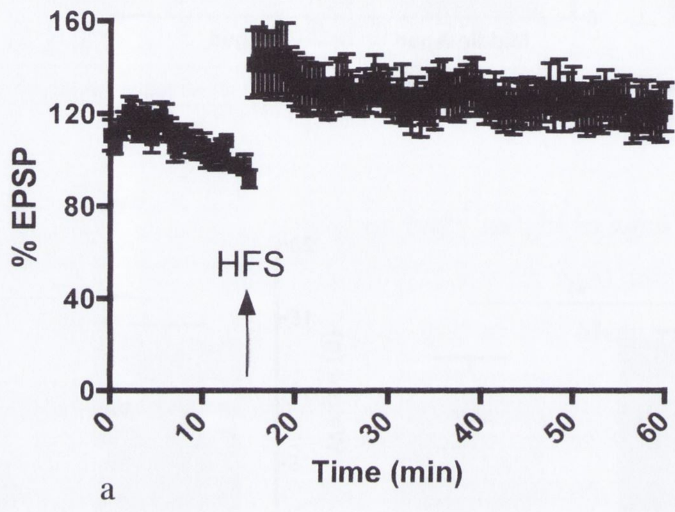


Figure 5.4a LTP in young control animals

Figure 5.4a shows mean data (± SEM) for each test shock delivered pre and post HFS. Data for young control animals (n=8) are presented.

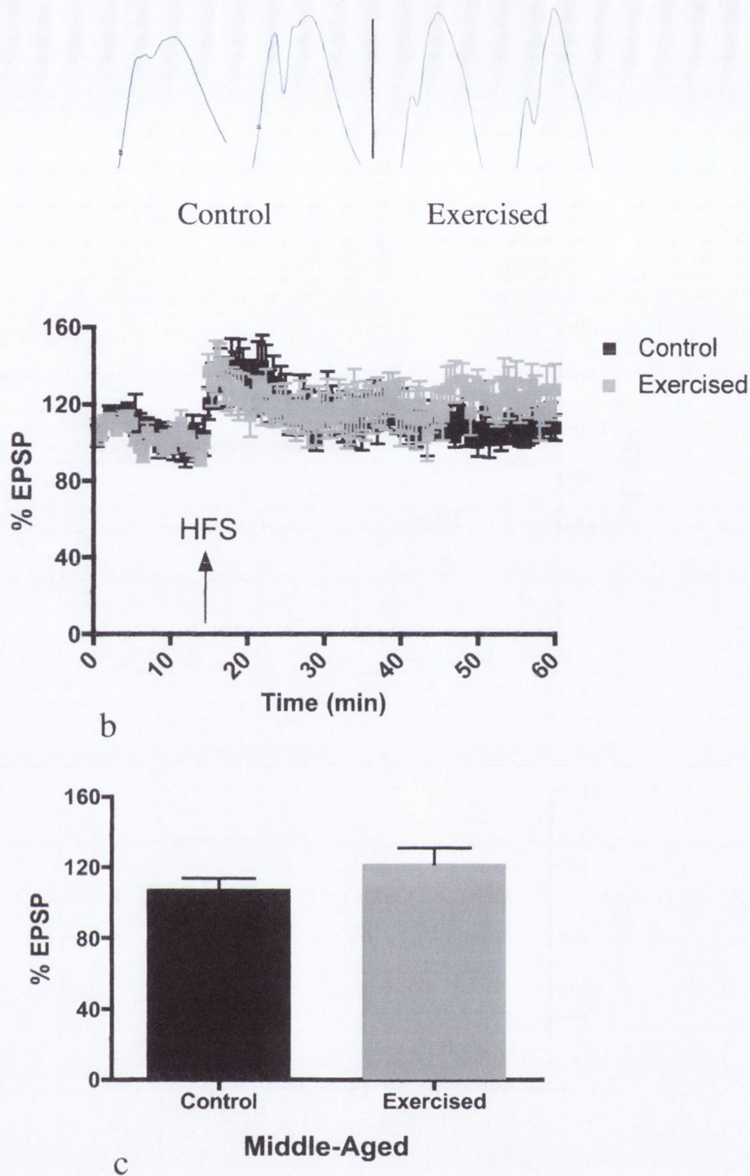


Figure 5.4b & c The effect of chronic exercise on LTP in middle-aged animals
Figure 5.4b shows mean data (± SEM) for each test shock delivered pre and post HFS; a 2-way ANOVA was used to analyse data post tetanus. Figure 5.4c c shows mean values (± SEM) for % EPSP during the last 5min of the experiment for control (n=6) and exercising (n=6) groups of middle-aged animals; an unpaired Student's t-test was used to assess the effect of exercise on LTP. There was no significant effect of chronic exercise on LTP in middle-aged animals.

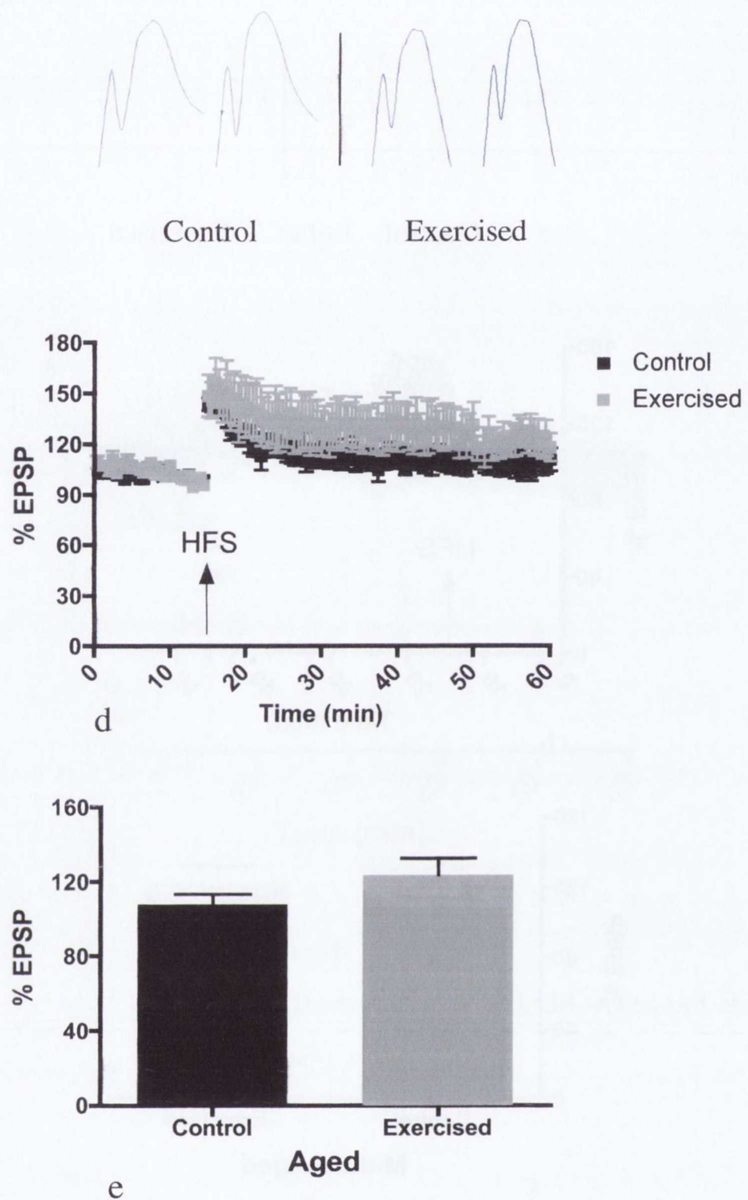


Figure 5.4d & e The effect of chronic exercise on LTP in aged animals

Figure 5.4b shows mean data (± SEM) for each test shock delivered pre and post HFS; a 2-way ANOVA was used to analyse data post tetanus. Figure 5.4c shows mean values (± SEM) for % EPSP during the last 5min of the experiment for control (n=5) and exercising (n=7) groups of middle-aged animals; an unpaired Student's t-test was used to assess the effect of exercise on LTP. There was no significant exercise effect in aged animals.

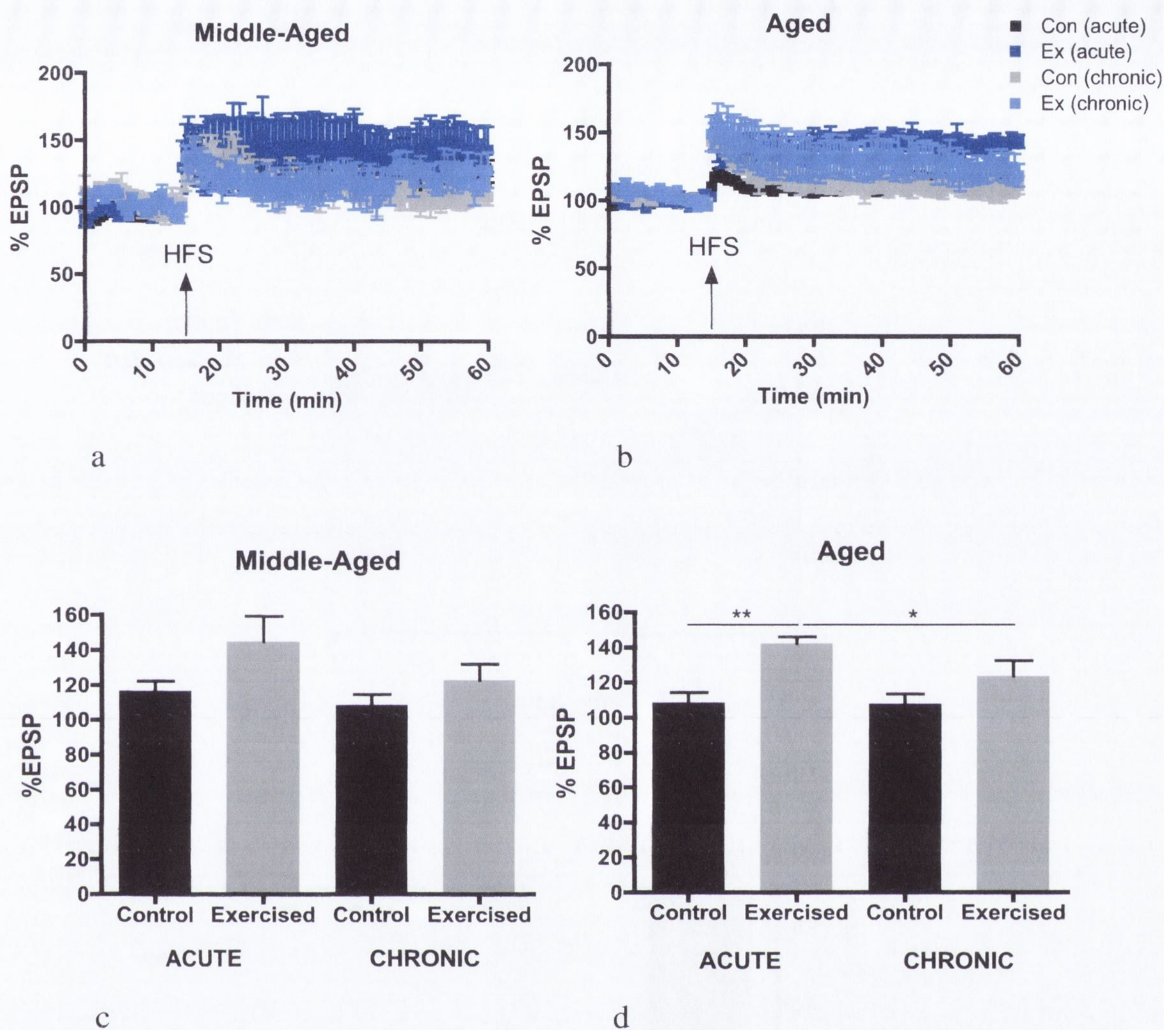


Figure 5.5a, b, c d b The effect of acute and chronic exercise on LTP

Data for control (con) and exercising (ex) groups of middle-aged rats (acute studies; con n=5, ex n=5 and chronic studies; con n=6, ex n=6) and aged rats (acute studies; con n=6, ex n=6 and chronic studies; con n=5, ex n= 7) from acute and chronic exercise are shown. Analysis by 2-way ANOVA revealed an effect of acute and chronic exercise on LTP in aged animals (Figure 5.4b; $p<0.05$). Figure 5.5c & d show mean values (± SEM) for % EPSP during the last 5min of the experiment; a 1-way ANOVA was used for statistical analysis. In aged animals, acute exercise produced an enhancement in LTP ($p<0.01$). There was a significant difference in LTP expression between exercised rats that completed the chronic exercise program and exercised rats that completed the acute program ($p<0.05$).

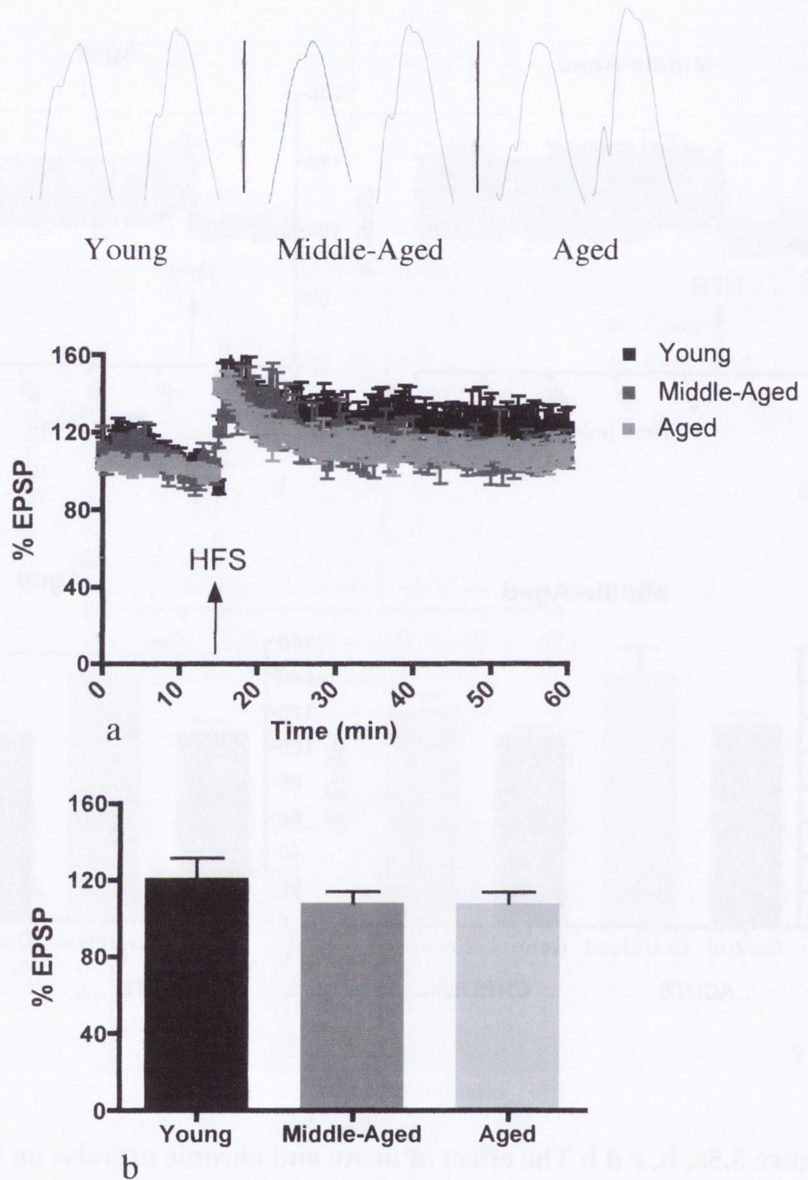
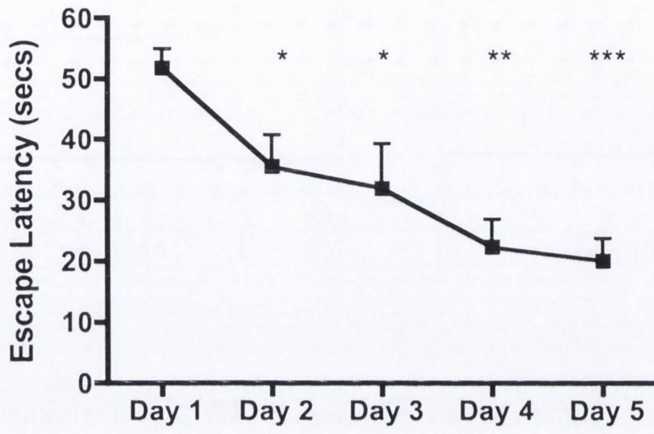


Figure 5.6a & b The effect of age on LTP

Figure 5.6b shows mean data (± SEM) for each test shock delivered pre and post HFS. A comparison of young, middle-aged and aged animals was completed by 2-way ANOVA. Figure 5.6b shows mean values (± SEM) for % EPSP during the last 5min of the experiment; a 1-way ANOVA was used for statistical analysis. There were no significant differences between young (n=8), middle-aged (n=6) and aged (n=5) animals suggesting that there was no effect of age on LTP.



a

Figure 5.7a MWM performance in young control animals

Results for mean escape latency, which represents MWM performance, for young control animals (n=8) are shown. Results are given as mean values (± SEM), for statistical analysis a 1-way ANOVA and post hoc Neuman-Keuls test was used. There were significant differences on day 2 ($p<0.05$), day 3 ($p<0.05$), day 4 ($p<0.01$) and day 5 ($p<0.001$) when compared with day 1 that suggests young animals learnt the task.

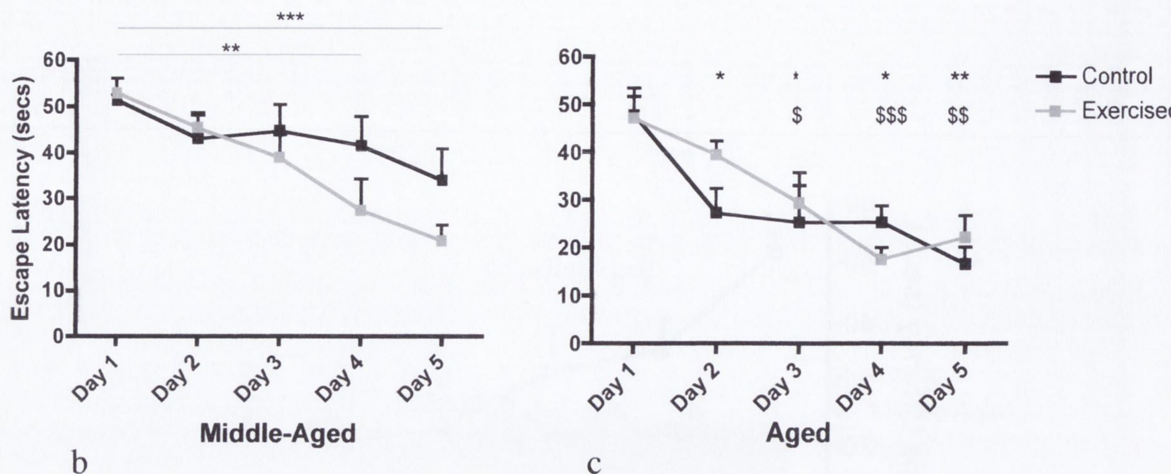


Figure 5.7b & c The effect of chronic exercise on MWM performance

Escape latency data for middle-aged (control n=5, exercised n=6) and aged (control n=6, exercised n=6) animals are presented. Results are given as mean values (± SEM). To identify if there was an effect of day on escape latency data from separate groups of rats were analysed by 1-way ANOVA. To identify if there was an effect of day or exercise on escape latency data from control and exercised age-matched groups were analysed by 2-way ANOVA. For middle-aged exercising animals there was a significant decrease in escape latency on day 4 ($p<0.01$) and day 5 ($p<0.001$) when comparing performance with that on day 1. Because control animals did not similarly learn the task, it is presumed that chronic exercise had a positive effect on spatial learning. For aged animals, there was no effect of chronic exercise on escape latency, but there was a significant effect of day on escape latency that suggests learning occurred in both control and exercising groups. Significant changes are denoted with the symbol * for control animals. Significant changes are denoted with the symbol $ for exercising animals. Control animals show evidence of learning on day 2 (escape latency was significantly less than that on day 1; $p<0.05$) where exercised animals demonstrate evidence of learning on day 3 (escape latency was significantly less than day 1; $p<0.05$).

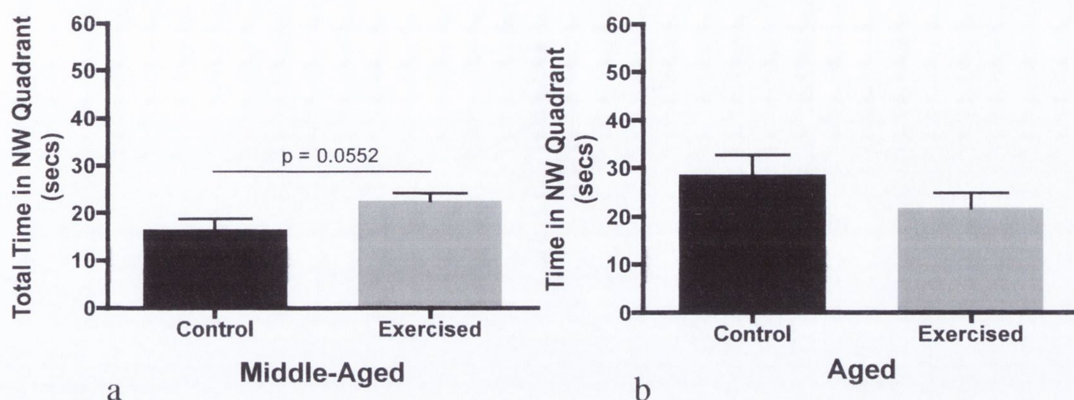


Figure 5.8a & b The effect of chronic exercise on probe test performance

Results for time spent in the NW pool quadrant during the probe test for middle-aged and aged control and exercised animals are shown. Data are given as mean values (± SEM), for statistical analysis an unpaired Students' t-test was used. There was no difference for time swimming in the NW quadrant between control (n=5) and exercised (n=6) animals in the middle-aged group (p=0.052). Similarly, there was no difference in time swimming in the NW quadrant between control (n=6) and exercised (n=6) animals in the aged group.

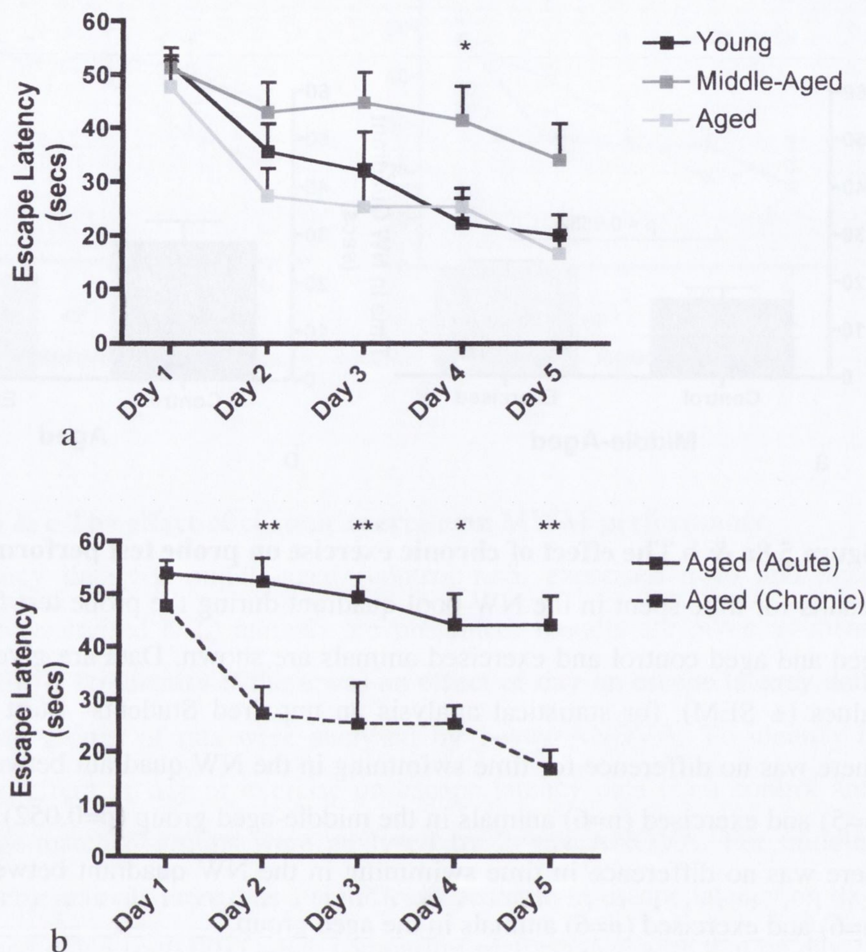
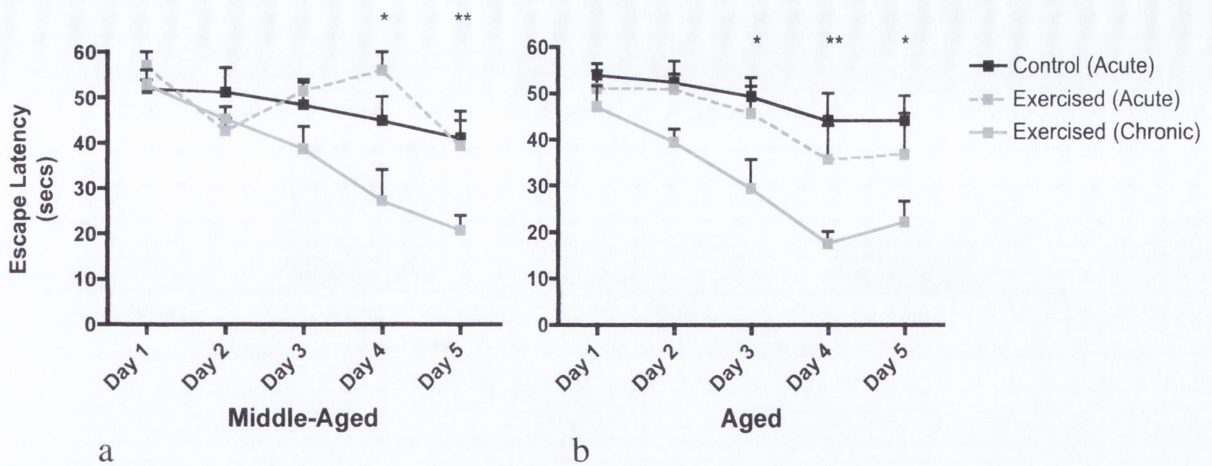


Figure 5.8a & b The effect of age on spatial learning (a) and MWM performance in aged control animals; a comparison of acute and chronic studies (b)

Escape latency data (mean ± SEM) are presented for control animals in young (n=8), middle-aged (n=5) and aged (n=6) populations. On day 4 of the task middle-aged animals were significantly slower ($p<0.05$) at finding the escape platform than both young and aged animals (Figure 5.8a), which suggests there is an age-related impairment in middle-aged, but not aged rats. Figure 5.8b shows results for aged control animals from acute (n=6) and chronic (n=6) studies. There were significant differences between groups on day 2 ($p<0.01$), day 3 ($p<0.01$), day 4 ($p<0.05$) and day 5 ($p<0.01$). This is a surprising result and suggests that aged control animals used in the chronic studies were not naïve.



a b

Figure 5.10a & b The effect of acute exercise and chronic exercise on MWM performance

Figure 5.10a & b show the effect of acute and chronic exercise on escape latency in the MWM task for middle-aged (control n=5, acute exercise group n=2, chronic exercise group n=6) and aged (control n=6, acute exercise group n=6, chronic exercise group n=6) animals. Results are given as mean values (± SEM), for statistical analysis a 2-way ANOVA with post hoc Neuman-Keuls was used. Control rats from acute studies (age-matched naïve animals) are compared with exercised groups. For middle-aged animals that had completed the chronic exercise program escape latency on day 4 and 5 was significantly less than day 1 ($p<0.05$ and $p<0.01$ respectively). This result suggests that chronic exercise, but not acute exercise, improved learning. For aged animals that had completed the chronic exercise program escape latency on day 3, day 4 and day 5 was significantly less than on day 1 ($p<0.05$, $p<0.01$ and $p<0.05$ respectively). Similar to the middle-aged group, this result suggests that chronic exercise, but not acute exercise, improves spatial learning.

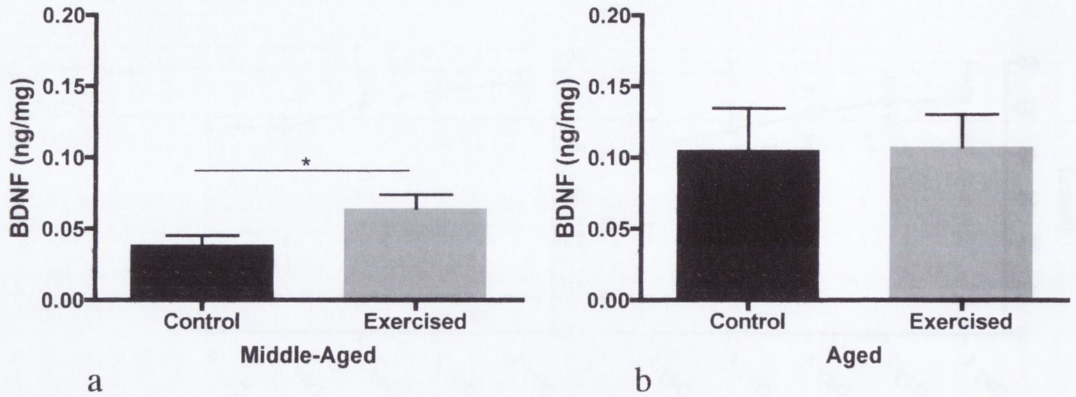
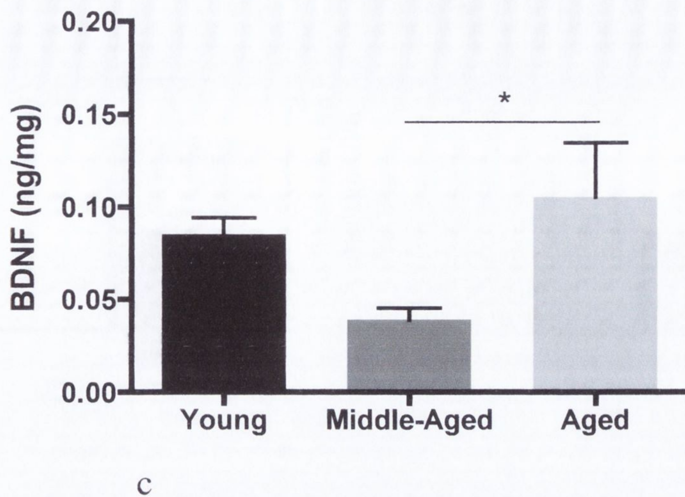


Figure 5.11a & b The effect of chronic exercise on the concentration of BDNF
Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was an exercise-induced increase in the concentration of BDNF in the dentate gyrus for middle-aged animals ($p<0.05$; control n=11, exercised n=12). For aged animals (control n=11, exercised n=13) BDNF concentration in the dentate gyrus was not different between control and exercised groups.



c

Figure 5.11c The effect of age on BDNF concentration

Results for BDNF concentration in the dentate gyrus of young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis. BDNF concentration was greater in aged rats compared with middle-aged rats ($p<0.05$).

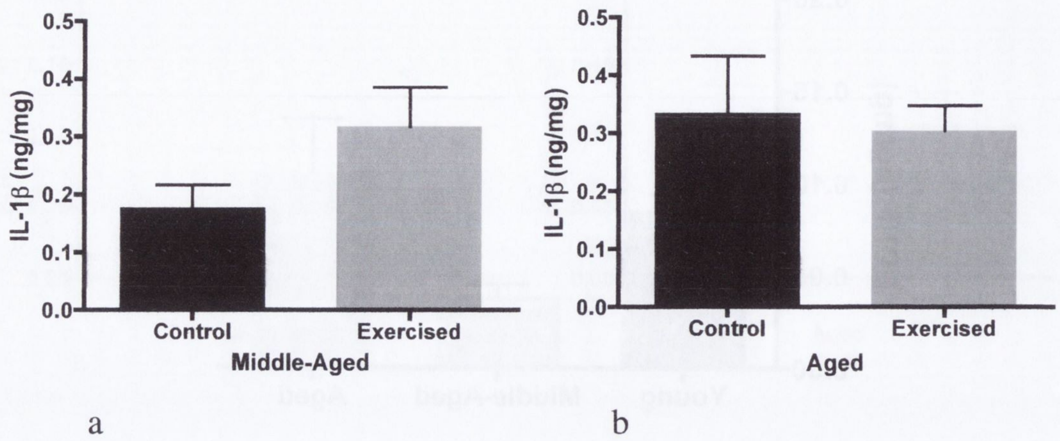
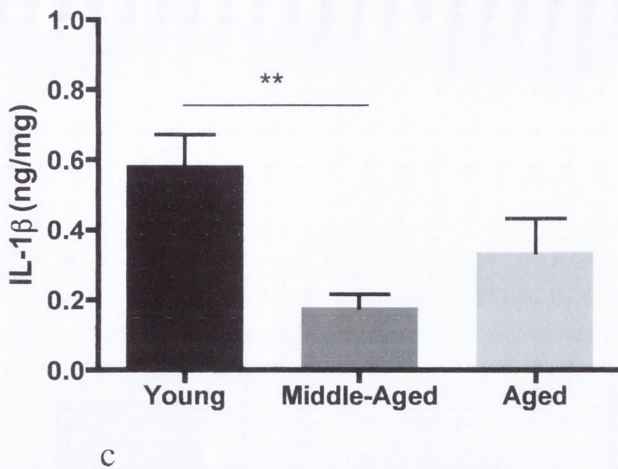


Figure 5.12a & b The effect of chronic exercise on IL-1β concentration

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was no exercise-induced effect on the concentration of IL-1β in middle-aged (control n=11, exercised n=12) or aged (control n=11, exercised n=13) animals.



c

Figure 5.12c The effect of age on IL-1β concentration

Results for the concentration of IL-1β in young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis. There is evidence of an age-related decline in IL-1β concentration. The concentration of IL-1β is significantly less in middle-aged rats compared with young rats ($p<0.01$).

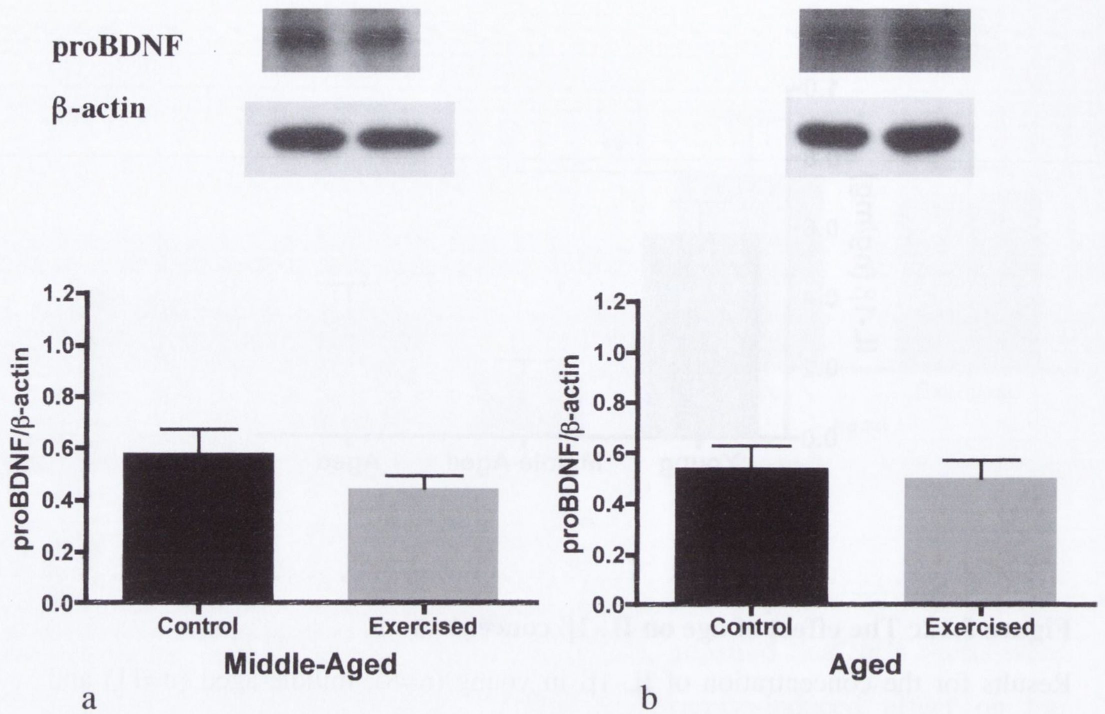
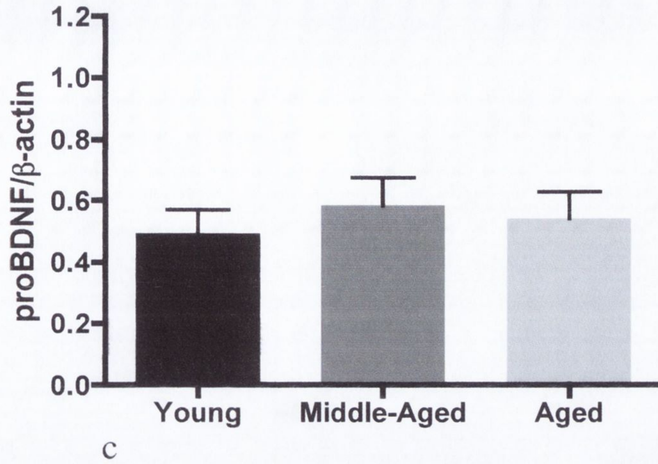


Figure 5.13a & b The effect of chronic exercise on proBDNF expression

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no exercise-induced effect on proBDNF expression in the dentate gyrus of middle-aged (control n=11, exercised n=12) or aged (control n=11, exercised n=13) animals.



c

Figure 5.13c The effect of age on proBDNF expression

Results for proBDNF expression in the dentate gyrus of young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis.

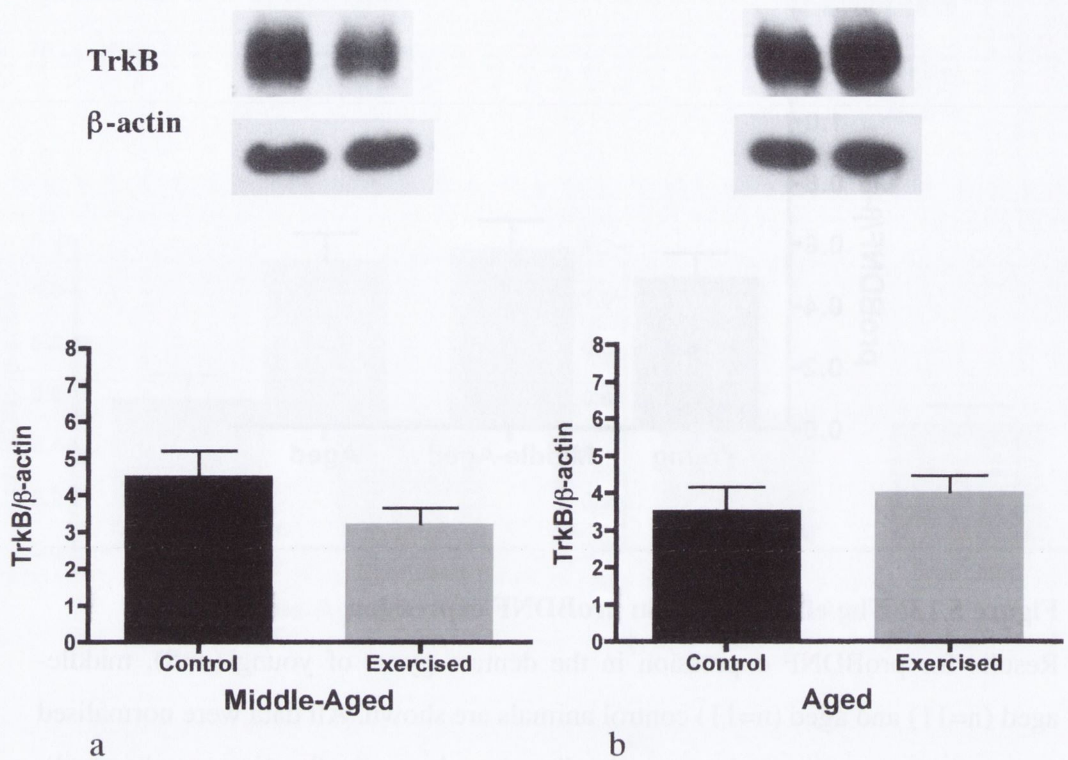
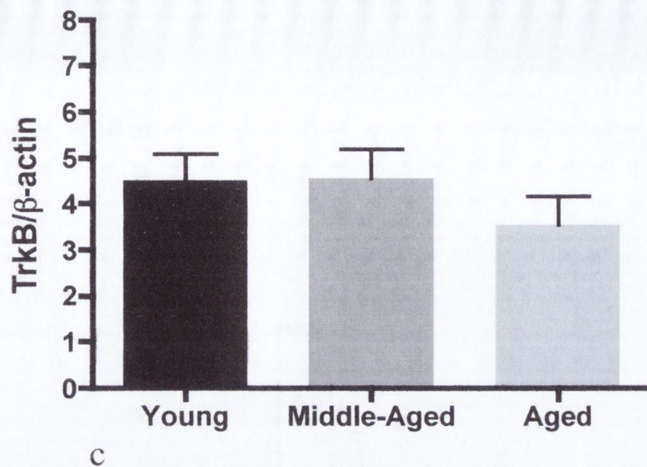


Figure 5.14a & b The effect of chronic exercise on the expression of TrkB

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no effect of chronic exercise on TrkB expression in middle-aged (control n=11, exercised n=12) or aged (control n=11, exercised n=13) animals.



C

Figure 5.14c The effect of age on TrkB expression

Results for TrkB expression in the dentate gyrus of young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis. There was no effect of age on the expression of TrkB.

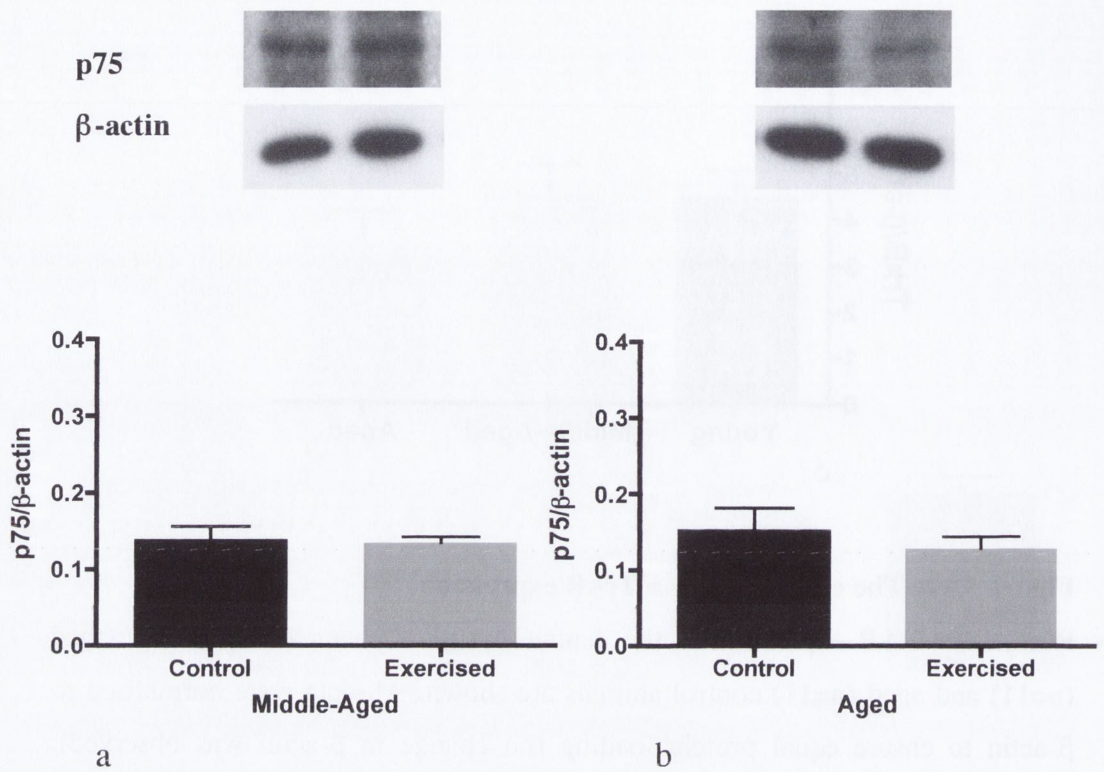


Figure 5.15a & b The effect of chronic exercise p75 expression

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). There was no exercise-induced effect on p75 expression in middle-aged (control n=11, exercised n=12) or aged (control n=11, exercised n=13) animals.

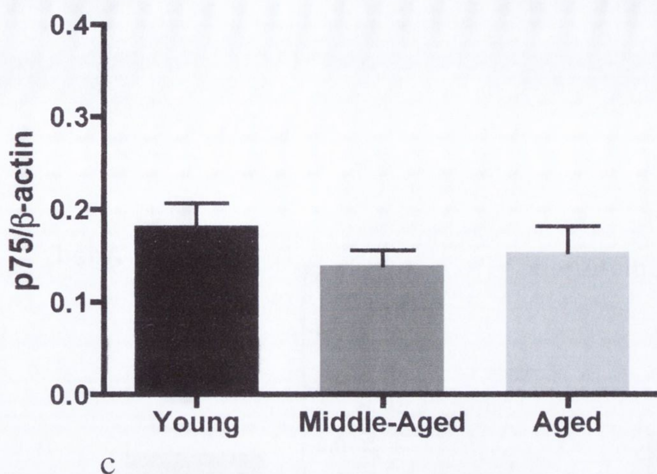


Figure 5.15c The effect of age on p75 expression

Results for p75 concentration in the dentate gyrus of young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. All data were normalised to β-actin to ensure equal protein loading (no change in β-actin was observed). Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis. There was no effect of age on p75 expression in the dentate gyrus.

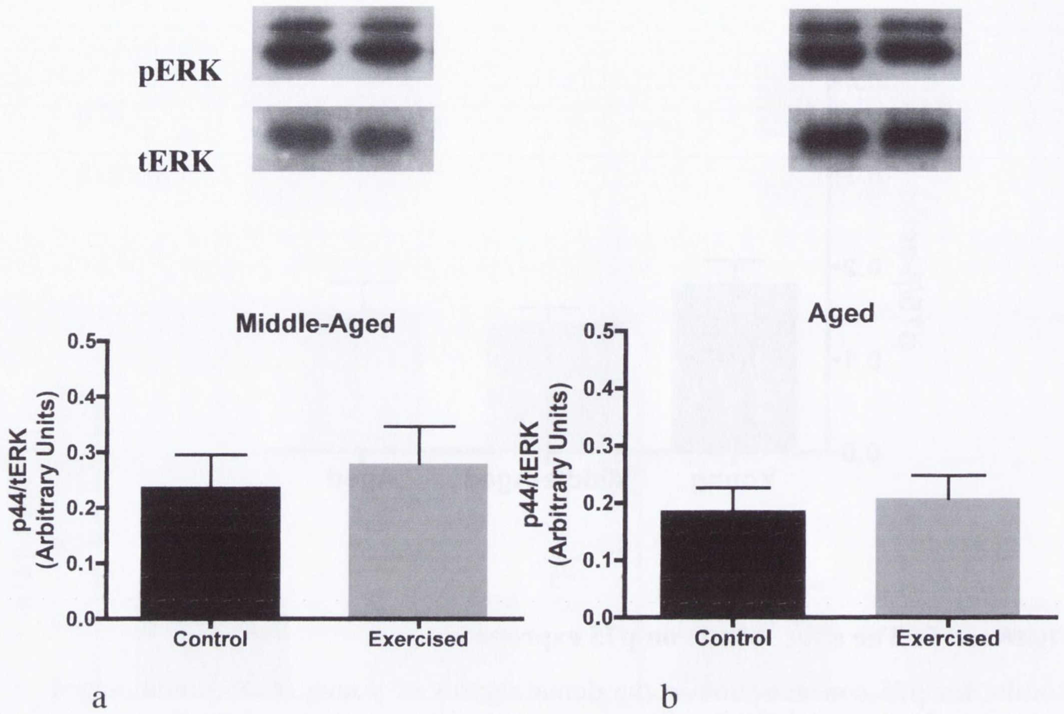


Figure 5.16a & b The effect of chronic exercise on ERK activation

Results are expressed as mean values (± SEM), unpaired Student's *t*-tests were used for statistical analysis. There was no exercise-induced effect on ERK activation in middle-aged (control n=11, exercised n=12) or aged (control n=11, exercised n=13) animals.

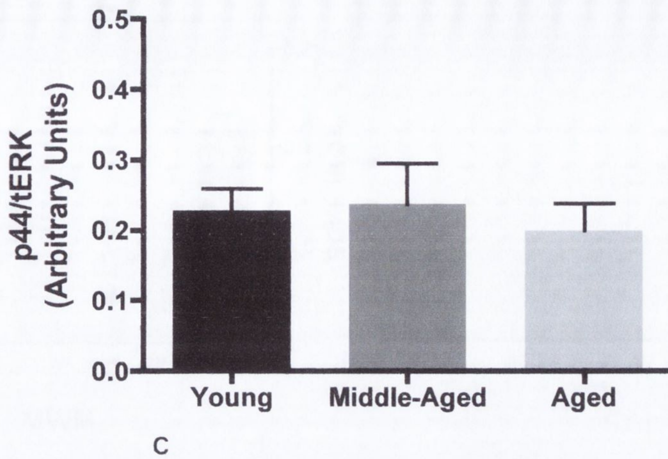
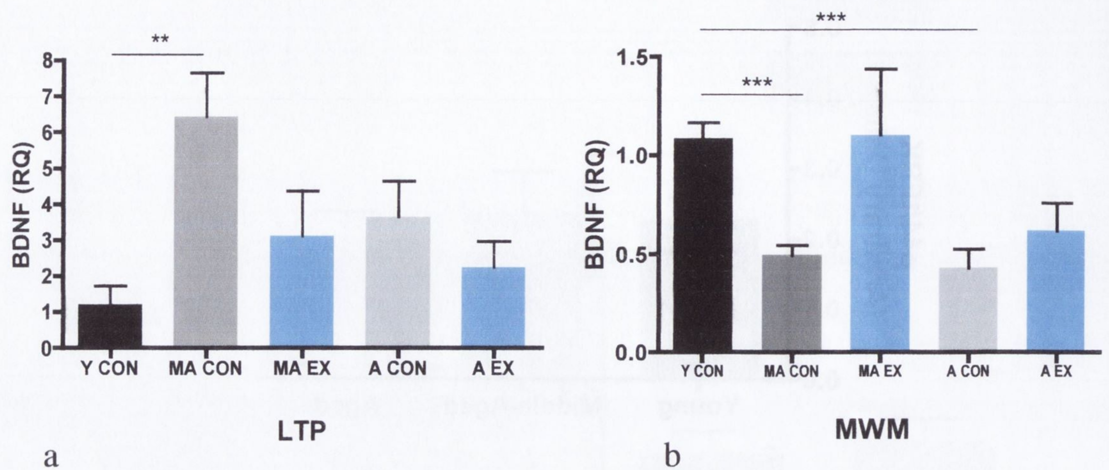


Figure 5.16c The effect of age on ERK activation

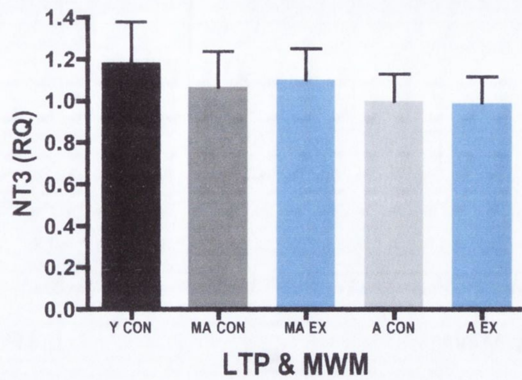
Results for ERK activation in the dentate gyrus of young (n=8), middle-aged (n=11) and aged (n=11) control animals are shown. Results are expressed as mean values (± SEM), a 1-way ANOVA was used for statistical analysis. There was no effect of age on ERK activation in the dentate gyrus.



a b

Figure 5.17a & b The effect of chronic exercise and age on BDNF mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. In LTP experiments there was a significant increase in BDNF mRNA in middle-aged control compared with young control animals ($p<0.01$). For MWM studies, BDNF mRNA was significantly reduced with age ($p<0.001$).



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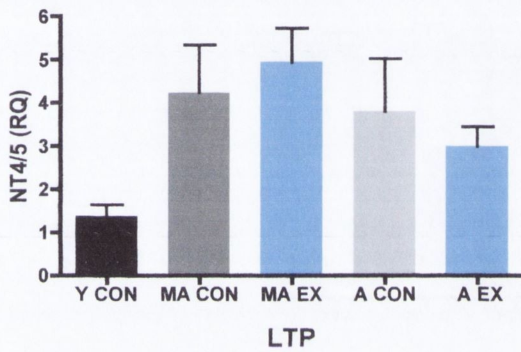
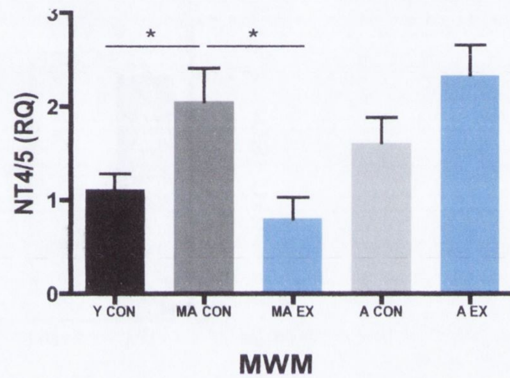


b



c

Figure 5.18a, b & c The effect of chronic exercise and age on NT3 and NT4/5 mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. There was no effect of chronic exercise or age on NT3 mRNA (Figure 5.18a). For LTP experiments there was no effect of chronic exercise or age on NT4/5 mRNA (Figure 5.18b). For MWM experiments (Figure 5.18c) there was a significant decrease in NT4/5 in middle-aged exercising animals compared with age-matched controls ($p<0.05$). In addition, there was an increase in NT4/5 in middle-aged control animals compared with young control animals ($p<0.05$).

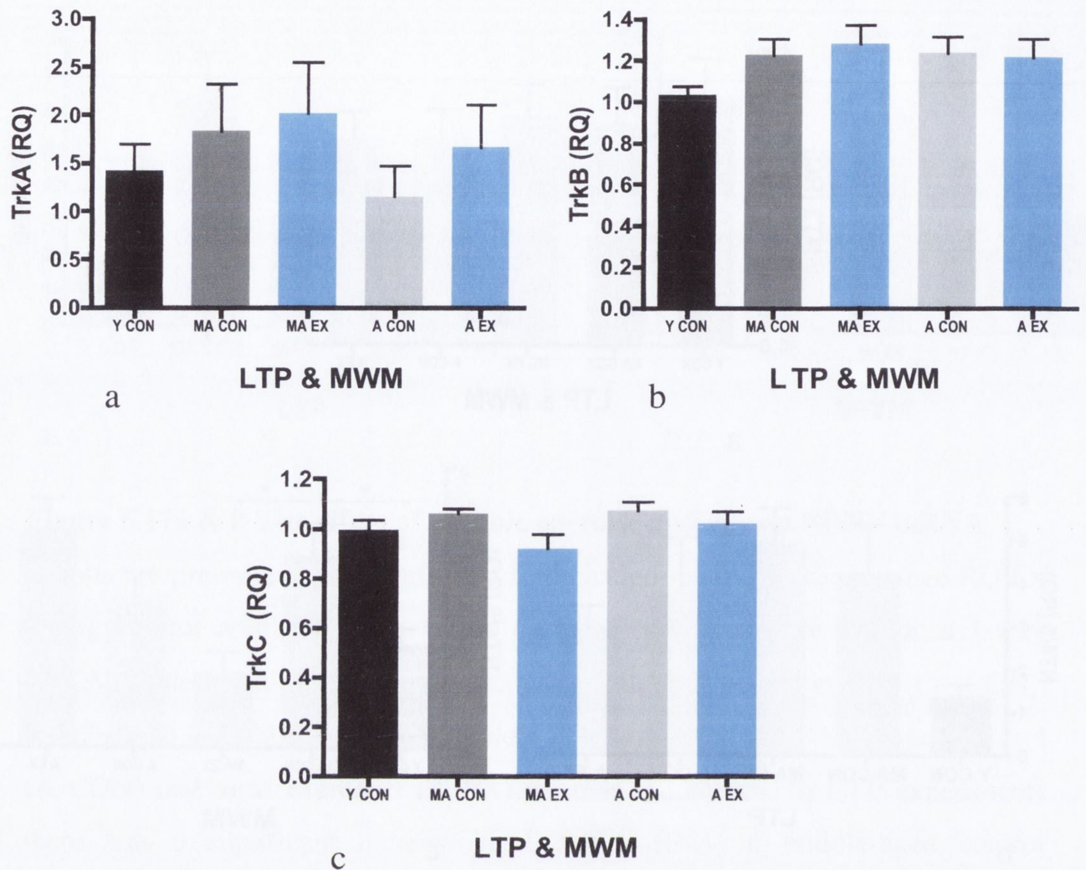


Figure 5.19a, b & c The effect of chronic exercise and age on TrkA, TrkB and TrkC mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. There was no effect of chronic exercise or age on mRNA of any of the tyrosine kinase receptors.

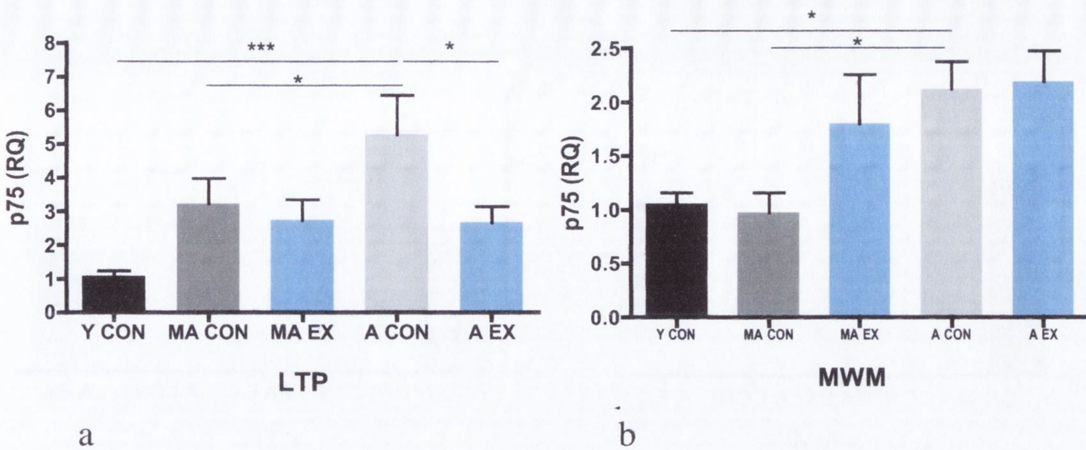


Figure 5.20a & b The effect of chronic exercise and age on p75 mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. For LTP experiments (Figure 5.20a) there was a significant decrease of p75 mRNA in aged exercising animals compared with age-matched controls ($p<0.05$). In addition there was a significant increase in p75 mRNA with age ($p<0.001$ for young and aged control animals, $p<0.05$ for middle-aged and aged animals). For MWM experiments (Figure 5.20b) there was also an age-related increase in p75 mRNA ($p<0.05$ for young and aged control animals, $p<0.05$ for middle-aged and aged animals).

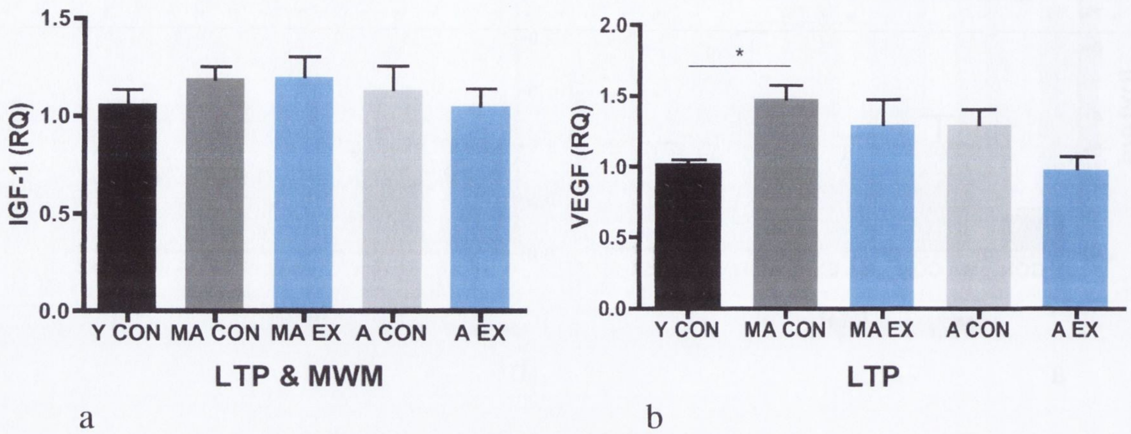


Figure 5.21a & b The effect of chronic exercise and age on IGF-1 and VEGF mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. There was no effect of exercise or age on IGF-1 mRNA in the dentate gyrus. For MWM experiments there was no effect of exercise or age on VEGF mRNA. However, there was an age-related increase in VEGF mRNA in middle-aged control animals compared with young control animals in LTP experiments ($p<0.05$).

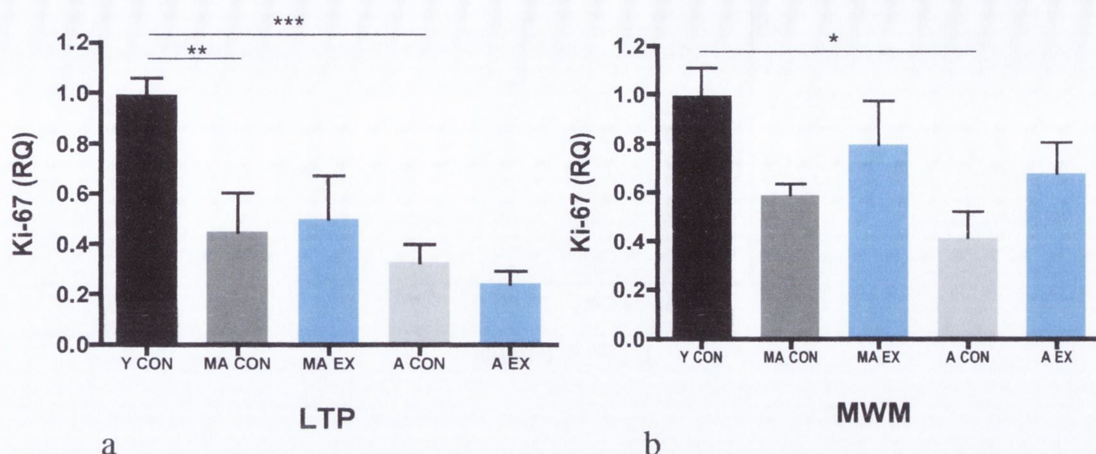


Figure 5.22a & b The effect of chronic exercise and age on Ki-67 mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. In LTP studies there was a progressive decline in Ki-67 mRNA with age ($p<0.05$; young and middle-aged control animals, $p<0.001$; young and aged animals). In the MWM studies there appears to be a trend for an exercise-induced increase in Ki-67 in both middle-aged and aged animals but these differences were not found to be significant. Similar to the LTP studies there was an age-related decline in Ki-67 mRNA when comparing young and aged animals in the MWM studies ($p<0.05$).

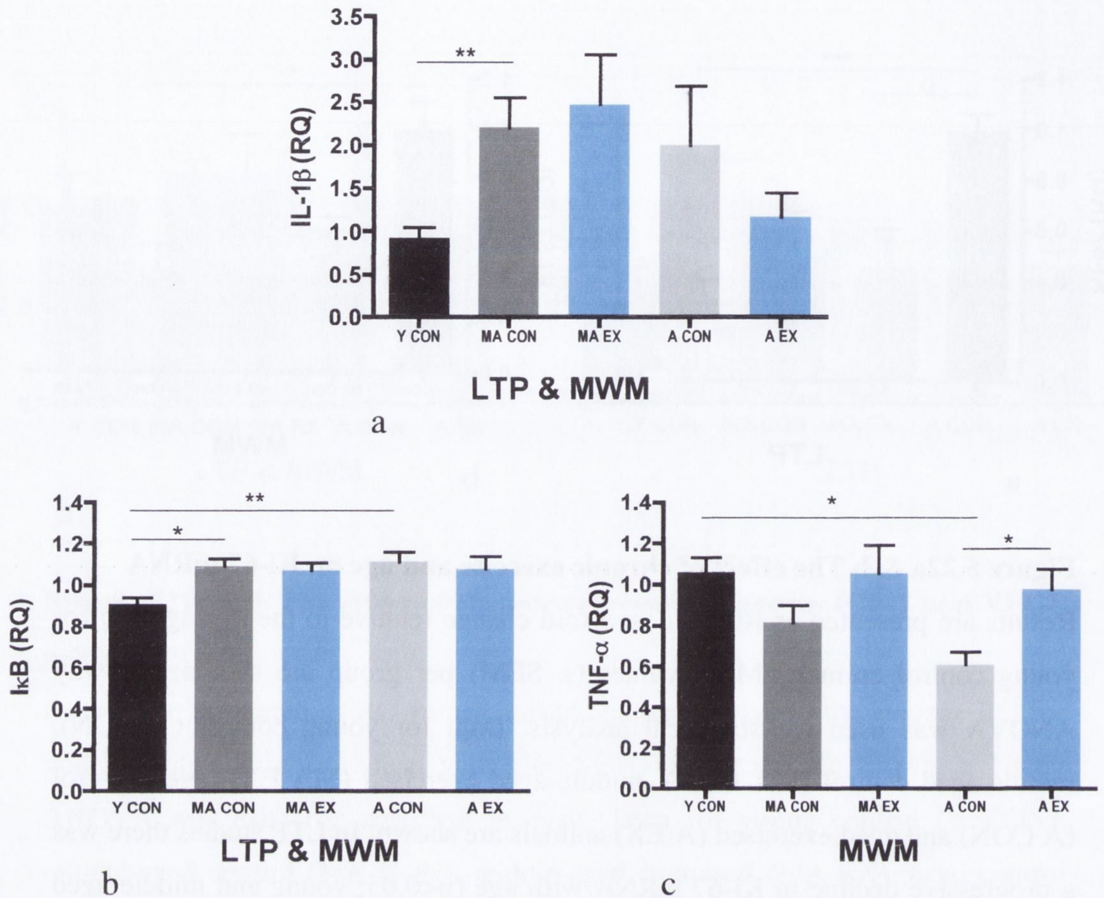


Figure 5.23a b & c The effect of chronic exercise and age on IL-1β, IκB and TNF-α mRNA

Results are presented as RQ values; a fold change relative to the average RQ for young control animals. Mean values (± SEM) per group are shown; a 1-way ANOVA was used for statistical analysis. Data for young control (Y CON), middle-aged control (MA CON), middle-aged exercised (MA EX), aged control (A CON) and aged exercised (A EX) animals are shown. There is a trend for an exercise-induced decrease in IL-1β in aged animals (not significant). There was an age-related increase in IL-1β. Middle-aged animals had significantly more IL-1β mRNA than young control animals ($p<0.01$) and there is a similar trend in aged animals when compared with young (not significant). The results for IκB show a progressive increase in IκB mRNA with age ($p<0.05$; young compared with middle-aged animals, $p<0.01$; young compared with aged animals). Interestingly, there was no effect of chronic exercise or age on TNF-α mRNA in

LTP studies, but was an exercise-induced increase in TNF-α mRNA in aged animals ($p<0.05$) and an age-related decline when comparing young and aged control animals in MWM studies ($p<0.05$).

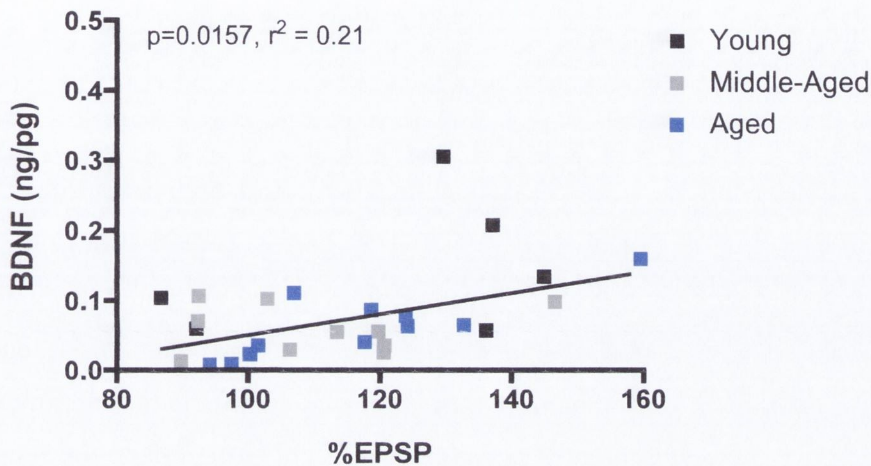


Figure 5.24 The correlation between LTP and BDNF concentration in the dentate gyrus

The relationship between LTP and BDNF concentration in the dentate gyrus is shown. Data from all animals used in the chronic exercise studies are presented. Statistical analysis was completed by linear regression with a confidence interval of 95%. The mean value for % EPSP during the last 5min of the experiment following tetanic stimulation gives an indication of the ability of rats to sustain LTP. There was a positive correlation between % EPSP and concentration of BDNF protein in the dentate gyrus ($p=0.0157$, $r^2 = 0.21$).

5.4 Discussion

The main aim of this study was to investigate the effects of a chronic 8-month exercise program on LTP and spatial learning in differently aged populations of animals and to explore the underlying cellular mechanisms involved. Analysis was completed to determine whether acute and chronic exercise programs have different effects on cognitive function and to identify whether the mechanisms mediating these effects are similar or specific to the duration of exercise. Finally, age-related changes in hippocampal function were explored.

These experiments show that chronic exercise can positively impact hippocampal function and that the potential for exercise to improve cognitive function is not dependent on age. An exercise-induced enhancement in spatial learning was observed following an 8-month exercise program in middle-aged and aged animals. This effect was associated with an increase in BDNF protein in middle-aged animals. In aged animals the positive effect of exercise on the brain was associated with a reduction in p75 mRNA in the dentate gyrus. There was also a trend for an exercise-induced increase in the proliferative marker Ki-67 mRNA which supports the theory that neurogenesis may underlie exercise-induced improvements in hippocampal function. In addition, age-related increases in IL-1β mRNA were someway abrogated by chronic exercise in aged rats allowing speculation that exercise may have an anti-inflammatory effect. Although the changes in Ki-67 and IL-1β were not significant, they provide further insight into the variety of mechanisms by which exercise affects brain function.

Collectively, the results indicate that acute and chronic exercise programs may have differential effects on hippocampal function. Acute exercise robustly enhanced LTP in young and aged animals, but did not improve spatial learning. In contrast, the most significant effect of chronic exercise was seen in relation to spatial learning.

Considering age-related changes, there was a decline in spatial learning with age. An-age related decrease in hippocampal weight (expressed as a percentage of brain weight) was observed. Additionally, age–related impairments in hippocampal function were associated with an increase in p75 mRNA, a decrease in Ki-67 mRNA and an increase in the inflammatory markers IL-1β mRNA and IκB mRNA in the dentate gyrus.

5.4.1 The effect of exercise on body weight

The benefits of exercise to general health are well-documented. The body weight of all animals used in the chronic exercise studies was increased over the 8-month experimental period. There was evidence that exercise reduced body weight in the aged group suggesting that general health was improved in this population. It is a natural presumption that long-term aerobic exercise will reduce body weight. Indeed, it is well recognised that in human populations the long-term effects of aerobic exercise include a reduction in body weight, body mass index and body fat (McTiernan *et al.*, 2007).

5.4.2 The effect of exercise on LTP

Although the results from these studies reveal a trend for an increase in LTP following chronic exercise, these changes were not significant. Previous research has shown that exercise may enhance LTP, however the majority of this research has focused on young populations of rodents (Christie *et al.*, 2005; Farmer *et al.*, 2004; van Praag *et al.*, 1999), or rodents exercising for shorter periods than that of the present studies. Van Praag and colleagues (2005) is the only known study to have investigated the effects of exercise on hippocampal function in aged rodents. Here, an improvement in hippocampal plasticity was observed following a month of voluntary exercise in aged mice. It is clear that more research is needed to confirm whether the exercise-induced enhancement in LTP is consistently matched in aged animals.

Comparing the results from acute exercise studies with those from chronic exercise studies it appears that acute exercise has the potential to more robustly enhance LTP than chronic exercise. This is an interesting result and supports the theory that improvements in hippocampal function may be specific to the exercise program undertaken. It is speculated that where short periods of exercise have the potential to improve some forms of hippocampal function such as synaptic plasticity, extended exercise programs are required to demonstrate improvements in learning that presumably depend on a number of different cellular modifications. Alternatively, it has been proposed that the stress produced by forced exercise may negatively impact the potential for exercise to improve hippocampal function (Moraska *et al.*, 2000). Although this argument has been

challenged somewhat (Albeck *et al.*, 2006; Ang *et al.*, 2006), it is possible that when an exercise program is commenced the initial stress response interferes with exercise-induced cellular modifications and limits improvements in hippocampal function. With exercise programs of longer duration it is likely that an adaptation to exercise occurs allowing more marked improvements in hippocampal function to be demonstrated. Leeuwenburgh and Heinecke (2001) suggest that adaptations to exercise may decrease oxidative stress by a number of mechanisms including an increase in antioxidant defences.

5.4.3 The effect of exercise on spatial learning

Previous research suggests that exercise-induced improvements in spatial learning are produced more reliably with prolonged exercise programs. Focusing specifically on aged populations of rodents, van Praag and colleagues (2005) observed an improvement in spatial learning following 45 days voluntary wheel running, Nichol and colleagues (2007) demonstrated an improvement in the radial arm maze with 3 weeks of voluntary wheel running, and Albeck and colleagues (2006) showed an enhancement in spatial learning after 7 weeks of mild forced treadmill exercise. Although there are some studies that report no effect of exercise on spatial learning, this inconsistency may be explained by the variability in study design. The results of the current experiments show an enhancement in spatial learning following an 8-month chronic exercise program in middle-aged animals. For aged animals, the effects of chronic exercise in spatial learning were not as well defined. Following more detailed analysis of the data it is believed that the aged control group were not naïve animals. Firstly, the results for MWM performance of aged control animals were closely matched with young control animals. This is unusual, as an age-related decline in spatial learning would be expected, similar to that observed with the acute studies. Secondly, when comparing control aged animals from acute and chronic studies, there were significant differences between the groups. If both these groups represented naïve animals, similar patterns of learning would be expected. Given that the aged control animals used in the chronic studies were handled regularly, and were frequently exposed to a stationary treadmill over the 8-month experimental period, it is proposed that this treatment constituted a form of environmental

enrichment. Environmental enrichment has been associated with an enhancement in hippocampal neurogenesis in aged rats that has been linked with an improvement in spatial learning (Nilsson *et al.*, 1999). To identify whether chronic exercise had an affect on spatial learning in this age group and to account for the possibility that environmental enrichment had improved MWM performance in control animals, aged exercising animals were compared with age-matched naïve animals (control animals from acute studies). This analysis revealed an exercise-induced enhancement in spatial learning.

Although the explanation for enhanced spatial memory in aged control animals used in the chronic studies is entirely plausible, it is interesting that environmental enrichment did not appear to affect spatial learning in the middle-aged control group. An age-related decline in MWM performance was observed that would indicate environmental enrichment had no effect on spatial learning in middle-aged rats. Previous research has shown that older populations of animals are most responsive to the positive effects of environmental enrichment on hippocampal function. Harburger and colleagues (2007) investigated the age-dependent effects of environmental enrichment on spatial reference memory in male mice. The results revealed that 24hr/day environmental enrichment for approximately 6 weeks significantly improved spatial memory in the MWM in aged, but not in young or middle-aged groups. Additionally, Frick and colleagues (2003) suggest that environmental enrichment initiated at middle age can reduce age-related impairments in spatial memory in mice. These studies support the contention that aged animals are most responsive to environmental enrichment.

To draw conclusions, an enhancement in spatial learning following chronic exercise in middle-aged and aged rats has been demonstrated. Comparing these results with those from acute exercise studies, the findings clearly support the body of literature reporting an improvement in spatial learning with prolonged exercise. In addition, it is speculated that spatial learning was enhanced in aged control animals used in the chronic exercise studies as a result of environmental enrichment.

5.4.4 The effect of exercise on neurotrophin induction

BDNF has been strongly linked to improvements in cognitive function.

Correlation analysis of the current data revealed that BDNF protein in the dentate gyrus was associated with an increase in LTP expression. This result supports Korte and colleagues (1996) who report a functional role for BDNF in the expression of LTP in the hippocampus. In addition, it has been demonstrated that compromised spatial learning may be protected by BDNF administration (Radeki *et al.*, 2005), and that impaired spatial learning and memory may be caused by a down-regulation of BDNF mRNA expression in the hippocampus (Li *et al.*, 2005).

In the context of exercise, although it has been frequently reported that BDNF plays a key role in mediating the effects of exercise on cognitive function (Berchtold *et al.*, 2005; Chen *et al.*, 2005; Vaynman *et al.*, 2004b), it is important to acknowledge that a number of other mechanisms may also be involved in producing the effects of exercise on the brain. An exercise-induced change in BDNF concentration in the dentate gyrus of middle-aged animals, but not aged animals, was observed in the current studies. A point that was previously raised in the context of an exercise-induced increase in hippocampal BDNF was that most studies reporting this link have observed these changes in young animals (Chen *et al.*, 2005; Vaynman *et al.*, 2003). It is also important that previous studies have used exercise regimes of shorter duration, ranging from 3 days to 2 weeks, than that used here and may explain why similar changes were not observed. Interestingly, BDNF protein in aged control rats was similar to that in young control rats, which may support the theory that aged control rats were responsive to environmental enrichment. Indeed, Rossi and colleagues (2006) reported that BDNF is required for the enhancement of hippocampal neurogenesis following environmental enrichment. If aged control animals had elevated levels of BDNF this may have masked an exercise effect. Ideally experiments should be repeated using a naïve group of aged rats, which would enable dissociation of an exercise effect and an environmental enrichment effect on hippocampal function and cellular parameters.

Regardless of the explanations given for the current results the findings do lend support to the theory that exercise may mediate its effects on cognition through an up-regulation of BDNF. However, it is likely that this mechanism is not solely responsible and additional factors are involved in mediating the effects of exercise

on hippocampal function.

Interestingly, an exercise-induced decrease in NT4/5 mRNA in the dentate gyrus of middle-aged animals in MWM experiments was observed. Being the most recently identified neurotrophin there is little information regarding its expression and function. That said, the few studies that have investigated the role of neurotrophin 4/5 suggest it plays a role in the potentiation of developing synapses (Wang & Poo, 1997), hippocampal physiological and morphological plasticity (Schwyzer *et al.*, 2002) and pyramidal cell neuroprotection (Royo *et al.*, 2006). Similar to BDNF, NT4/5 signals predominantly through the TrkB receptor and previous research collectively suggests that its actions promote cell survival. Further research is warranted to fully elucidate the role of NT4/5 in mediating the effects of exercise on hippocampal function.

5.4.5 The effect of exercise on TrkB and p75

There was no effect of chronic exercise on TrkB protein or TrkB mRNA in the dentate gyrus in either middle-aged or aged animals. Although previous research has demonstrated exercise-induced increases in TrkB expression these effects have mostly been observed in young animals exercising for less than 2 months (Klintsova *et al.*, 2004; Widenfalk *et al.*, 1999).

There was no effect of chronic exercise on p75 protein expression in the dentate gyrus however, an exercise-induced decrease in p75 mRNA in the dentate gyrus of aged animals was observed in LTP experiments. This is especially interesting given that an exercise-induced decrease in the expression of p75 protein was observed in aged animals following acute exercise. It is surprising that consistent changes in protein and mRNA expression were not always observed. An explanation for these differences may be that changes in the expression of a protein, and changes in the expression of mRNA of that same protein, might be mismatched due to timing. That is, the time taken to translate the genetic code via the mRNA intermediate into a functional protein must be considered. In addition, because protein homogenate was analysed, changes in protein expression could not be localised. A consequence of this could be that protein changes specific to a particular region were masked by analysing a change in whole homogenate. Nonetheless, it is promising that there is a similar pattern for exercise-induced

changes in p75 within this age group and it is speculated that the mechanisms involved in mediating the effects of exercise on brain function are age-dependent. There is very limited research exploring the possibility that the p75 neurotrophin receptor is involved in mediating the effects of exercise on cognitive. Greferath and colleagues (2000) demonstrated that spatial learning performance was consistently better in p75 knockout mice than controls. Moreover, Yaar and colleagues (1997) produced data that implied neuronal death in Alzheimer's disease is mediated, to some extent, by the interaction of amyloid-β with the p75 neurotrophin receptor. Indeed, studies that were previously completed in this laboratory have shown that lipopolysaccharide (LPS), an endotoxin that promotes the secretion of pro-inflammatory cytokines, induces impairments in LTP and recognition memory that are associated with an increase in expression of the p75 neurotrophin receptor in the dentate gyrus, thus supporting the theory that p75 negatively affects plasticity (Hennigan *et al.*, 2007). Additionally, blockade of p75 with the neutralising antibody, REX, enhances LTP and spatial learning, suggesting that this receptor negatively regulates synaptic plasticity (Áine Kelly, personal communication). Taken collectively, these studies emphasize the negative effects of p75 activation on cognitive function, thus it may be hypothesised that an exercise-induced decrease in the expression of p75 mRNA may accompany an improvement in cognitive function. Further investigation is required to more clearly understand the involvement of p75 in mediating an exercise-induced effect on hippocampal function. In light of the current results, it is speculated that changes in the p75 protein and or gene in response to exercise may be limited to aged animals.

5.4.6 The effect of exercise on IGF-1 and VEGF

It has recently been reported that a key mechanism mediating the effects of exercise on the brain involves the induction of central and peripheral growth factors and associated cellular cascades that ultimately produce structural and functional change (Cotman *et al.*, 2007). Although the role played by BDNF is probably the best investigated, both IGF-1 and VEGF have also been shown to contribute to the exercise-induced effects on cognition.

No effect of chronic exercise on the expression of IGF-1 mRNA in the dentate

gyrus was observed. Initially it was believed that an increase in circulating IGF-1 mediated the activational effects of exercise on the brain (Carro *et al.*, 2000) and was specifically associated with an increase in the number of new neurons in the hippocampus (Trejo *et al.*, 2001). More recently Ding and colleagues (2006) reported that the effects of exercise on brain neuronal and cognitive plasticity are modulated in part by a central source of IGF-1. Blocking hippocampal IGF-1 in young rats abolished the effect of exercise (5 days voluntary exercise) on memory recall, but did not significantly affect the ability of exercise to enhance acquisition in the MWM. It is difficult to directly compare the present results with those of Ding and colleagues (2006), the only other group that has explored the effect of exercise on the expression of IGF-1 in the brain, because the study designs are so different.

There was no effect of chronic exercise on VEGF mRNA in the dentate gyrus in the current experiments. It has been suggested that peripheral VEGF is necessary for the effects of 7 days voluntary running on hippocampal neurogenesis in young mice (Fabel *et al.*, 2003). It has also been reported that cerebral angiogenesis can be induced in aged female rats (22 months) following 3 weeks of treadmill exercise, 30min per day (Ding *et al.*, 2006). These changes were associated with an increased expression of VEGF mRNA and VEGF protein. It may be the case, that the initial exercise-induced increase in VEGF is peripheral, and that this change is subsequently translated to the brain. Thus, exercise-induced changes within the brain particularly, may be time-dependent. This may explain why no significant exercise-induced effect in VEGF mRNA in the dentate gyrus was observed here. It would be interesting to analyse blood samples for levels of VEGF and identify whether an exercise-induced effect is observed. In any case, the effects of exercise on the cerebral vasculature of both young and aged rats and the importance of angiogenesis to improvements in hippocampal function merit serious experimental investigation, but were beyond the scope of the present study.

5.4.7 The effect of exercise on neurogenesis

The Ki-67 protein is a marker of cellular proliferation and the expression of Ki-67 gives an indication of the degree of neurogenesis. There is a large body of

evidence that has shown an exercise-induced increase in neurogenesis (Chen *et al.*, 2006; Rhodes *et al.*, 2003., van Praag *et al.*, 1999a; van Praag *et al.*, 2005). These effects have been demonstrated with both voluntary and forced exercise. Ra and colleagues (2002) observed an increase in cellular proliferation in the dentate gyrus of young rats engaged in treadmill running and swimming exercise programs. Exercise sessions were completed on 3 consecutive days; 30min treadmill exercise or 5min swimming sessions. The MWM experiments completed for this study revealed a trend for an exercise-induced increase in Ki-67 mRNA in the dentate gyrus of middle-aged and aged rats but these differences were not significant. It is important to recognize that the reported increases in neurogenesis in response to exercise have commonly been seen following short periods of exercise. Thus, one possible explanation for why there was no significant exercise-induced effect on the expression of Ki-67 mRNA here is the prolonged exercise period used. In addition, Kim and colleagues (2004) produced evidence that suggested the most robust enhancing effects of exercise on cell proliferation are seen in young rodents. Treadmill exercise (30min per day for 5 consecutive days, maximum speed of 8m/min) increased cell proliferation in the dentate gyrus of all age groups (4-week-old, 8-week-old and 62-week-old) but this exercise-effect was most prominent in 8-week-old rats. A final point for consideration is that if, as speculated, aged control animals used for chronic studies were responsive to environmental enrichment, this group may have a greater expression of Ki-67 than age-matched naïve animals. If this were the case, an exercise-induced effect on neurogenesis in this population could have been masked. Indeed, Segovia and colleagues (2006) demonstrated that neurogenesis in the dentate gyrus of both young and aged rats is increased by environmental enrichment.

5.4.8 The effect of exercise on inflammation

Cotman and colleagues (2007) recently suggested that a common mechanism underlying the central and peripheral effects of exercise may be related to inflammation, which can impair growth factor signalling both systemically and in the brain. IL-1β is a pro-inflammatory cytokine. Nuclear factor-κB (NF-κB) is a transcription factor that is involved in cellular responses to stimuli such as stress,

cytokines and free radicals. NF-κB activation is initiated by the signal-induced degradation of IκB proteins. Tumour necrosis factor-α (TNF-α) is a cytokine involved in systemic inflammation. The expression of these inflammatory markers was analysed in an attempt to identify whether chronic exercise had an anti-inflammatory effect thus providing further insight into the mechanisms involved in mediating exercise-induced changes within the aged brain. Although there was no significant positive exercise-induced effects on these inflammatory markers, there was a trend for an exercise-induced decrease in IL-1β mRNA in aged animals. Surprisingly, there was also an exercise-induced increase in TNF-α mRNA in this age group. Bagby and colleagues (1994) reported that exercise markedly suppresses the systemic TNF-α response that is normally observed with an LPS challenge. Kitamura and colleagues (2007) also observed an exercise-induced suppression of plasma TNF-α after LPS administration in female rats. Although previous research appears to conflict with the results presented here, it is important to appreciate that published research has shown changes in TNF-α in the periphery following a single bout of exercise, where changes in the expression of TNF-α mRNA in the dentate gyrus following an 8-month exercise program was analysed here.

5.4.9 The effect of age on the structure of the hippocampus

Results show an age-related decrease in the weight of the hippocampus (expressed as a percentage of whole brain weight), heart weight and liver weight. The most pertinent of these results is that of the hippocampus, which may suggest an age-related cell loss specific to this brain region. Indeed, magnetic resonance imaging (MRI) has shown a reduction of hippocampal volume with age that is associated with a decrease in neurogenesis (Driscoll *et al.*, 2006). The present results are in broad agreement with this, but for conclusive proof of age-related cell loss analysis of hippocampal cell number would be required.

5.9.10 The effect of age on LTP and spatial learning

Considering age-related changes in hippocampal function, there was no impairment of LTP with age, a similar result to that seen in acute studies. Possible explanations for this result have been discussed previously. In relation to age-

related changes in spatial learning an age-related decline in spatial learning is reported. Middle-aged control animals displayed clear signs of impaired spatial learning. On day 4 of the MWM task middle-aged animals had significantly longer escape latencies than young and aged control animals. This effect was not due to a difference in swim speed between groups. An age-related impairment in spatial learning was also observed in acute studies. These results are in concurrence with published research that has reported age-related impairments in spatial learning assessed by the MWM (Fordyce & Wehner, 1993; Geinisman *et al.*, 2004), the 8-arm radial arm maze (Shukitt-Hale *et al.*, 2004) and the Barnes maze (Greferath *et al.*, 2000).

5.9.11 The effect of age on cellular parameters

When identifying age-related cellular changes, a trend showing an age-related decrease in BDNF protein was observed when comparing young and middle-age rats, this was not significant. Interestingly, BDNF protein was similar in young and aged animals and a comparison of middle-aged and aged rats revealed a significant difference between these groups. The fact that aged rats had levels of BDNF similar to young rats provides further support to the theory that this group were responsive to environmental enrichment. The evidence suggests that environmental enrichment recovers the age-related loss in BDNF protein and enhances spatial learning. An age-related increase in BDNF mRNA was observed when comparing young and middle-aged animals used for LTP experiments. This result may seem unexpected, however, important points to consider are that a wide variety of stimuli may increase BDNF, and also, changes in BDNF may be transient. Additionally, the variation in BDNF mRNA across lifespan is not well researched. In contrast the MWM studies show an age-related decrease in BDNF mRNA. Hattiangady and colleagues (2005) produced evidence that BDNF protein concentration declines precipitously by middle-age (12 months) in the dentate gyrus, CA1 and CA3 subfields of the rat hippocampus. The only explanation that can be provided for the different LTP and MWM results is that the stress imposed by the MWM task had a more significant effect in aged animals and down-regulated BDNF.

An age-related increase in NT4/5 mRNA was seen in the dentate gyrus when

comparing young and middle-aged animals. Considering previous research and the knowledge that NT4/5 binds with high affinity to TrkB it may be hypothesised that an increased expression of this neurotrophin would positively affect the fate of the cell. However, the pattern of NT4/5 mRNA expression observed in response to exercise and age does not appear to support this theory. It is particularly difficult to interpret this result as there is no reference material for direct comparison.

Results for the neurotrophin receptor p75 show an age-related increase in p75 expression in the dentate gryus. Considering previous research, and given that the present results have revealed an age-related impairment in some forms of hippocampal function, an age-related increase in p75 expression may be expected. There is very little published research focusing specifically on p75, but this novel result suggests that age-related changes in p75 should be further investigated.

Given that neurodegenerative diseases may be related to an increase in vascular dysfunction (Lopez-Lopez *et al.*, 2004) it is relevant to consider whether changes in the expression of VEGF accompany the aging process. Shetty and colleagues (2005) reported that the concentration of hippocampal VEGF declines considerably by middle age but remains steady between middle age and old age in F344 rats. In contrast, the current results show an age-related increase in VEGF mRNA when comparing young and middle-aged animals. It would be interesting to investigate whether changes in VEGF protein match the pattern observed for this age-related change in VEGF mRNA. A thorough explanation for this result cannot be provided but it is speculated that other factors are accountable for the discrepancies between the current results and those of Shetty and colleagues (2005).

Considering age-related changes in Ki-67 mRNA expression, there was an age-related decrease in Ki-67 mRNA in the dentate gyrus that supports previous literature. It is well recognized that adult hippocampal neurogenesis declines precipitously with age (Kempermann *et al.*, 2002; Kim *et al.*, 2004; Segovia *et al.*, 2006).

Inflammation and its regulation by cytokines has been linked to many aspects of aging (Bodles & Barger, 2004). It has been reported that an interplay of pro-inflammatory and anti-inflammatory responses impact significantly on

hippocampal synaptic function in the aged rat (Nolan *et al.*, 2005). It is well-recognised that inflammatory changes accompanying the aging process may negatively impact brain function, in severe cases causing neurodegeneration. Age-related increases in IL-1β concentration in the cortex and hippocampus have been coupled to age-related deficits in LTP (Martin *et al.*, 2002b). Cacabelos and colleagues (1994) demonstrated that demented patients had an increase in IL-1β production in the central nervous system; the most marked changes were observed in brain regions where Alzheimer's disease neuropathology is most prominent. Indeed, there has been acknowledgement that IL-1β plays an important role in hippocampal synaptic function and may trigger apoptotic changes in the aged brain causing detrimental changes that are associated with neurodegenerative diseases (Lynch, 2002). In addition, there is evidence that supports a direct role of TNF-α in the pathogenesis of a number of age-associated diseases including Alzheimer's disease (Bruunsgaard & Pedersen, 2003). The data produced by these experiments demonstrates an age-related increase in the expression of IL-1β mRNA and IκB mRNA and supports the evidence that brain inflammation is a natural part of the aging process. Interestingly, an age-related reduction in TNF-α mRNA was observed when comparing young and aged animals. It is important to appreciate that previous research exploring changes in TNF-α in relation to disease, exercise or an LPS challenge have commonly identified peripheral and not central effects and may explain the different results.

5.4.12 Summary

In summary, these experiments have shown an improvement in spatial learning in middle-aged and aged animals following an 8-month forced exercise program. This exercise effect was associated with an increase in BDNF in the dentate gyrus in middle-aged animals. For aged animals, an exercise-induced down-regulation in p75 mRNA in the dentate gyrus was observed. No exercise-induced effects on the growth factors IGF-1 mRNA and VEGF mRNA were seen in the dentate gyrus but it is proposed that changes in the expression of these growth factors are initiated in the periphery. Interestingly, results from MWM studies indicate a trend for an exercise-induced increase in Ki-67 mRNA, this result warrants further investigation. With particular relevance to an aged population, it is also

possible that anti-inflammatory effects are contributing to exercise-induced effects on learning and memory.

It is very promising that exercise-induced improvements in cognitive function have been observed in aged animals following both acute and chronic exercise. Evidence suggests that exercise-induced effects on hippocampal function may be dependent on the duration of exercise. It is clear that there are many cellular mechanisms involved in mediating the effects of exercise on the brain. These mechanisms appear to be very complex and may also be dependent on a variety of different factors. Given the limitations of the current experiments this interplay of mechanisms could not be fully explored.

In relation to age-related changes in hippocampal function, a significant age-related decline in spatial learning is reported. Hippocampal weight (expressed as a percentage of brain weight) was reduced with age and suggests a structural change. For the group of aged control animals used in these studies there was an increase in BDNF protein in the dentate gyrus that was associated with improved spatial learning; it is speculated that this group were responsive to environmental enrichment. Other age-related changes include an age-related increase in p75 mRNA, IL-1β mRNA and IκB mRNA and an age-related reduction in Ki-67 mRNA. Collectively, these results suggest that aging is associated with increased expression of inflammatory markers concomitant with a decline in neurogenesis.

Chapter 6

General Discussion

Chapter 6

6.1 Discussion

The aging process is accompanied by a general decline in many physiological systems e.g. endocrine, cognitive and motor. The hippocampus is a brain structure that is important for higher cognitive functions, possesses a high degree of plasticity and is particularly vulnerable to the aging process (Burke & Barnes, 2006). Consequently, the aged hippocampus displays various structural and functional deficits such as alterations in mechanisms of neural plasticity and impairments in hippocampal-dependent learning. With severe cognitive decline Alzheimer's disease and other forms of dementia show symptoms of hippocampal dysfunction and cognitive alteration (Small *et al.*, 1999). Given that demographic trends suggest the elderly population is increasing (Miller & O'Callaghan, 2005), it is especially important that interventions with the potential to improve cognitive functioning or ameliorate neurodegenerative decline are thoroughly investigated. Research has shown that in addition to improving general health and being associated with numerous benefits to the cardiovascular system, exercise positively affects neuronal function and has the capacity to improve cognitive function. Thus, exercise may be viewed as a simple means of maintaining brain function and promoting brain plasticity (Cotman & Berchtold, 2002). It is well recognized that neurotrophins, most prominently BDNF, are central to the exercise-induced effects on brain function. Although this area of research has been extensively studied, the precise mechanisms that underlie an exercise-induced improvement in cognitive function have yet to be identified. In addition, evidence suggests that caution must be exercised in assessing the reported effects of exercise on cognitive function and the mechanisms mediating these effects; while there may be some generic effects of exercise on the brain, some effects are likely to be specific to exercise intensity, exercise duration and other factors.

The main aims of this research were to investigate the effects of exercise on hippocampal function in young, middle-aged and aged rodent populations. A comparison of the effects of acute and chronic exercise programs on cognitive function was completed to determine whether the benefits of exercise are

dependent on the duration of exercise. Moreover, experiments were conducted to identify what molecular mechanisms were involved in mediating these changes and consequently provide a better understanding of exercise-induced improvements in cognitive function.

6.2 Can exercise enhance hippocampal function?

There are numerous published reports that suggest exercise may enhance cognitive function (Anderson *et al.*, 2000; Farmer *et al.*, 2004; van Praag *et al.*, 1999). The research presented here clearly shows that exercise has the potential to enhance some forms of hippocampal function. An enhancement of LTP and recognition memory was observed in young rats following an acute forced exercise program. Exercising rats engaged in daily 1hr exercise sessions for 7 consecutive days. Given that improvements in hippocampal function in young rats in response to acute exercise were not consistent i.e. there was no improvement in spatial learning with this group, it is speculated that exercise may not induce cognitive enhancement in every measure of cognitive function. While successful expression of LTP in the dentate gyrus, spatial learning in the MWM and recognition memory have all been shown to depend on robust hippocampal function, each of these paradigms is likely to recruit different populations of neurons within the hippocampal formation and associated cortical regions. For example, the perirhinal cortex has been implicated in recognition memory. The observed specificity of acute exercise effects in young rats to LTP and recognition memory, but not spatial learning, may reflect exercise-induced enhancements in the function of neurons whose activity is required for LTP in the dentate gyrus and for recognition memory, but not for spatial learning. Alternatively, it may be the case that, with spatial learning in the MWM, a 'ceiling effect' exists, whereby the escape latency observed in the control group is of such short duration that an exercise-effect is not revealed unless a more challenging assessment of spatial learning is used such as that used by van Praag and colleagues (1999b). In studies of aged animals, where an improvement in spatial learning with chronic exercise is seen, it may be assumed that exercise-induced improvements are more easily observed where a measurable deficit exists. In view of these results, it is proposed

that exercise may not always induce cognitive enhancement but more consistently offers neuroprotection against neurodegenerative decline.

6.3 A neuroprotective role of exercise

Kiraly and Kiraly (2005) cited that both basic animal models and clinical data overwhelmingly support the hypothesis that exercise is a major protective factor against neurodegeneration of varied etiologies. Indeed, there is a strong body of evidence that suggests exercise acts to confer neuroprotection.

Gobbo and O'Mara (2005) investigated whether exercise could provide protection after brain damage specifically in terms of recovery of cognitive function. A kainic acid model of neuronal injury was used. Exercise, prior to kainic acid brain damage, improved behavioural performance in the MWM and object exploration tasks. Interestingly, although exercise substantially increased BDNF in the dentate gyrus, it did not prevent against neuron loss in CA1, CA2 and CA3 regions of the hippocampus. This result may indicate that neurogenesis in specific hippocampal subfields is not necessary for spatial learning or object exploration. Chen and colleagues (2006) also support a role for exercise in neuroprotection by reporting that moderate exercise enhances dentate gyrus neurogenesis and reverses the deficits in learning and memory in hippocampal-lesioned adult rats. In addition, Li and colleagues (2004) subjected young rats to transient (with reperfusion) or permanent middle cerebral artery occlusion (MCA) and observed that 3 weeks of exercise pre-conditioning reduced brain damage in rats subjected to transient MCA occlusion. The authors claim that this result demonstrates an exercise-induced endogenous neuroprotection, and that exercise preconditioning may be used as an effective intervention to reduce ischemia/reperfusion brain injury from stroke.

Where exercise positively impacts cognitive function in aged animals that demonstrate impaired hippocampal-dependent learning it may also be assumed that exercise is conferring neuroprotection. In the current studies there is evidence that spatial learning deficits in both middle-aged and aged animals were attenuated by chronic exercise. Similarly, Radak and colleagues (2001) reported that the age-associated decline in performance of the passive avoidance test that

represents both short and long-term memory was prevented by a 9-week swimming exercise program. Van Praag and colleagues (2005) observed an improvement in spatial learning of aged mice that engaged in voluntary wheel running for 45 days. Albeck and colleagues (2006) demonstrated that spatial learning was enhanced in aged rats engaging in mild forced treadmill exercise for 7 weeks. Interestingly, some studies have shown that age-related impairments in hippocampal-dependent learning are not ameliorated by exercise. Barnes and colleagues (1991) did not see an effect of endurance exercise on age-related impairments in spatial memory. Aged rats engaged in a 10-week exercise program using a motorised treadmill. Additionally, Hansalik and colleagues (2006) investigated the effects of voluntary and forced exercise on the age-related impairment in MWM behaviour and did not report any positive effect of voluntary or forced exercise on this from of learning.

The experiments completed here demonstrate evidence that exercise produces a neuroprotective effect of one form of hippocampal-dependent learning. Although there is a great body of literature advocating exercise as a neuroprotectant, it must be acknowledged that there is a degree of inconsistency amongst published reports.

An additional point for consideration is that despite the potential for exercise to improve cognitive function, either by enhancing plasticity or acting as a neuroprotectant, it is possible that because of an inherent variability in hippocampal function across populations, some animals and indeed some humans, may not respond to an exercise intervention as robustly as others. In earlier chapters the possibility of a natural variability in hippocampal synaptic plasticity with regard to age-related changes in LTP has been discussed. It is speculated that this concept may be equally relevant to exercise-induced changes.

6.4 Variability in Study Design

Where research reveals conflicting results it is possible that the origin of these differences lies in the large variations in study design. Surprisingly, there is little research that has directly focused on exploring the extent to which the variations in study design may affect exercise-induced changes within the brain. Kim and

colleagues (2003) reported that cell proliferation in the dentate gyrus of adult rats is modulated by the intensity and duration of exercise. The most marked cell proliferation was observed with light exercise and reached a maximum level after 7 days of exercise. Molteni and colleagues (2002) found a differential effect of acute and chronic exercise on plasticity related genes in the hippocampus. These studies both highlight that different exercise programs can produce different effects on the brain. Equally, the current experiments support the theory that the duration of exercise may be an important determinant in relation to the potential for exercise to enhance cognitive function i.e. these effects are exercise duration-specific. In further support of the theory that exercise duration is relevant to the exercise-induced effects on the brain, a recent review by Cotman and colleagues (2007) stated that although some studies show improvements in cognitive function after 1 week of exercise, most benefits have been associated with long-term exercise. Obviously further research is warranted to more clearly define the effects of exercise duration, intensity and mode on hippocampal function.

The studies completed here provide evidence that the effects of exercise may be specific to some forms of hippocampal function. Comments have already been made in regard young animals where improvements in LTP and recognition memory, but not spatial learning, were observed with acute exercise. In addition, results for aged animals revealed that exercise-induced effects may be specific to some forms of hippocampal function; improvements in LTP, but not spatial learning, were seen following acute exercise. Chronic exercise improved spatial learning in both middle-aged and aged animals but did not affect LTP. It is likely that exercise-induced effects on cognitive function, be they enhancing or protective, may (a) be specific to some forms of hippocampal function and (b) be exercise-specific.

6.5 Exercise-mediated mechanisms

There are a number of ways in which exercise may be mediating its effects within the brain. It has been suggested that alterations in hippocampal theta rhythm in response to exercise may contribute to improved learning (Baruch *et al.*, 2004). Cotman and Berchtold (2002) proposed that the medial septum, which provides a

source of cholinergic and GABAergic neurons to the hippocampus, is responsive to a persistent firing pattern (theta rhythm) induced by exercise and may be involved in up-regulating BDNF in response to exercise. Another mechanism by which exercise may affect brain function is an increase in brain perfusion. Timinkul and colleagues (2008) suggest that exercise may improve brain function by increasing cerebral blood volume (CBV). CBV was measured in the prefrontal cortex during graded cycling exercise to the point of exhaustion in young male subjects; CBV was increased at mild exercise intensities. In theory, increasing the delivery of nutrients to the brain may support neurogenesis and ultimately improve the integrity of brain regions associated with learning and memory. Alternatively, an antioxidant effect of exercise training may be responsible for exercise-induced neuroprotection (Ji, 2002).

Both acute and chronic exercise studies completed here have shown evidence that BDNF is, in some instances, involved in mediating the effects of exercise on the brain. In acute exercise studies, young exercising animals had an increased concentration of BDNF in the dentate gyrus when compared with age-matched controls. Specific to LTP and object recognition experiments, an exercise-induced BDNF up-regulation was observed in other brain regions associated with learning and memory. In addition, there was an up-regulation in BDNF in the dentate gyrus of middle-aged exercising animals when compared with age-matched controls. BDNF is the most widely distributed growth factor found within the brain and has a major influence on the survival and function of several neurotransmitter systems. There is a wealth of research that links BDNF to hippocampal function. Indeed, BDNF has an important functional role in the expression of LTP in the hippocampus (Korte *et al.*, 1996). Correlation analysis of the present data revealed a strong association between BDNF protein in the dentate gyrus and LTP sustainability. Chen and colleagues (2007) also demonstrated a link between BDNF and contextual learning. Given that BDNF is tightly linked to hippocampal-dependent learning, it is an obvious target for research investigating the mechanisms by which exercise mediates its effects on cognitive function. The theory that BDNF plays a central role in mediating the exercise-induced effects on brain plasticity is strongly supported (Berchtold *et al*,

2005; Molteni *et al*, 2002; Vaynman *et al*, 2003). Although the results from the current studies appear to support the contention that exercise-induced effects may be mediated by BDNF, there is no conclusive evidence that these effects are causal. For proof of a causal link between exercise-induced effects on cognitive function and BDNF the actions of BDNF would have to be blocked. If, by inhibiting BDNF, the positive effects of exercise on cognitive function are not observed, as Vaynman and colleagues (2004b) reported, one could clearly state that exercise employs the action of BDNF to enhance cognitive function.

If BDNF is indeed a key player underlying the effects of exercise on hippocampal function it is important to consider (a) how it is up-regulated, (b) where it is up-regulated and (c) how it is acting. It has been proposed that acetylcholine-mediated activation of the hippocampus may underlie the regulation of BDNF by exercise (Cotman & Berchtold, 2002). In relation to the source of an increase in the expression of BDNF the current results do not allow specific conclusions to be drawn. Gold and colleagues (2003) were the first to report that acute moderate exercise can induce an increase in BDNF serum concentrations in humans. Ferris and colleagues (2007) support this result and reported that, alongside improving cognitive function scores, serum BDNF levels were significantly increased in response to 30min exercise, and also that the magnitude of this response was intensity-dependent. Given that the blood brain barrier is permeable to neurotrophins (Poduslo & Curran, 1996) it is plausible that increases in peripheral BDNF may be translated to the brain. Alternatively, if the primary source of an exercise-induced elevation in BDNF is the brain, it is important to acknowledge that both types of brain cell, neurons and glia, are responsive to BDNF and must therefore equally be considered as a source of BDNF up-regulation. Todd and colleagues (2007) reported that neurotrophin-dependent modulation of neuron-glia signalling through differential mechanisms is mediated by NT3 and BDNF. Indeed, Hempstead and Salzer (2002) suggest that neurotrophins may be viewed as paracrine signals that mediate bidirectional signalling between neurons and glia rather than as factors that act on neurons alone. The analysis of BDNF concentration and expression in the dentate gyrus completed in these experiments did not enable specification of the source of an increase in BDNF.

In relation to the actions of BDNF and BDNF-mediated improvements in learning and memory, both pre-synaptic and post-synaptic mechanisms have been proposed. Xu and colleagues (2000) hypothesized that BDNF signalling modulates the competence of pre-synaptic neurons to generate the repetitive exocytotic events needed to modify the long-term responses of post-synaptic neurons. Tyler and colleagues (2002) speculated that both pre-synaptic and post-synaptic mechanisms may underlie the role of BDNF in learning and memory. Specifically within the context of exercise, Molteni and colleagues (2002) suggested that an exercise-induced elevation in the expression of BDNF can affect neuronal plasticity by acting at both pre- and post-synaptic terminals. The authors report that TrkB signalling at the pre- or post-synaptic terminal can result in up-regulation of several downstream genes, such as members of the MAPK pathway and CAMK family. With respect to pre-synaptic changes, it was proposed that exercise can modulate the expression of genes associated with synaptic trafficking to promote neurotransmitter release.

In the present studies investigations were completed to identify whether exercise-induced changes in BDNF were associated with alterations in the neurotrophin receptors TrkB and p75 or ERK activation. Interestingly, although there were exercise-induced changes in the neurotrophin receptors in aged animals with both acute and chronic exercise, these changes were not associated with alterations in BDNF concentration. There were no exercise-induced changes in ERK activation in the dentate gyrus. It is proposed that the timing of experiments did not allow exercise-induced alterations in ERK activation to be identified. In addition, given that binding of neurotrophins to their receptors stimulates several signalling pathways it is likely that an exercise-induced increase in BDNF could be acting via multiple signalling pathways to mediate its effects on hippocampal function. As an example, activation of the PI-3K/Akt pathway, which is linked to mechanisms of synaptic plasticity and memory consolidation (Horwood *et al.*, 2006), has been associated with exercise by Chen and colleagues (2005). Considering the present results and the relevant literature, it is believed that BDNF is an important player in mediating the effects of exercise on cognitive function. However, neither the source of an increase in BDNF nor its precise

mechanism of action can be specifically elucidated from the current experiments.

In further consideration of the mechanisms by which exercise mediates its effects it is also important to acknowledge that other growth factors, namely IGF-1 and VEGF, may contribute to the exercise-induced effects on the brain. Cotman and colleagues (2007) suggest that where the effects of exercise on learning are predominantly regulated by IGF-1 and BDNF, the exercise-induced stimulation of hippocampal neurogenesis and angiogenesis is regulated by IGF-1 and VEGF. Indeed, alternative to the theory that exercise-induced effects on cognition are primarily mediated by a neurotrophic effect, it is highly probably that angiogenesis may be central to the positive effects of exercise on the brain.

Exercise has repeatedly been shown to induce angiogenesis within the brain (Ding *et al.*, 2006; Isaacs *et al.*, 1992; Swain *et al.*, 2003). Of particular interest is a study by Pereira and colleagues (2007) that reported exercise has a "primary" effect on dentate gyrus CBV and may be correlated with changes in cognitive function. Additionally, considering the neuroprotective effect of exercise, Ding and colleagues (2006) attributed the beneficial effects of exercise pre-conditioning following imposed ischemic alterations in the brain to a strengthening of brain microvascular integrity.

In relation to neurogenesis, there is a large body of evidence demonstrating an exercise-induced increase in neurogenesis (Chen *et al.*, 2006; Rhodes *et al.*, 2003; van Praag *et al.*, 1999a; van Praag *et al.*, 2005). The current experiments demonstrate an age-related decrease in neurogenesis measured by the expression of Ki-67 mRNA that may be partly restored by exercise. Although it is plausible that BDNF mediates this exercise-induced enhancement in neurogenesis it is equally possible that angiogenesis is responsible for this effect. Fabel and colleagues (2003) suggest that peripheral VEGF, an important signalling protein involved in angiogenesis, is necessary for the effects of running on hippocampal neurogenesis. This study demonstrates a causal link between VEGF and exercise-induced neurogenesis. Peripheral blockade of VEGF abolished exercise-induced hippocampal neurogenesis but did not affect baseline neurogenesis in control animals. The authors propose that VEGF is an important "somatic regulator" of adult neurogenesis and may act independently of central mechanisms that are

commonly associated with hippocampal neurogenesis. Although there was no evidence of exercise-induced changes in VEGF mRNA in the dentate gyrus in the current studies, it is speculated that the initial increase occurs in the periphery and that this change is subsequently translated to the brain. Thus, exercise-induced changes within the brain particularly, may be time-dependent, which might explain why no significant exercise-induced effect in VEGF mRNA was observed. Interestingly, van Praag and colleagues (2005) demonstrated that a lack of angiogenesis in aged rats was not a rate-limiting factor for the exercise-induced increase in neurogenesis that was demonstrated.

There is strong evidence that inflammatory changes within the aging brain are associated with neurodegenerative disease (Cacabelos *et al.*, 1994; Lynch, 2002). The results showing an age-related increase in the expression of IL-1β mRNA and IkB mRNA in the dentate gyrus support the evidence that brain inflammation is a natural part of the aging process. Cotman and colleagues (2007) recently suggested that an anti-inflammatory effect of exercise might subserve its potential to improve cognitive function and ameliorate neurodegenerative decline. The authors propose that exercise, by causing a reduction in inflammation, effectively reduces peripheral risk factors that may cause cognitive decline and neurodegeneration by interfering with mechanisms of growth factor signalling. In further support of an anti-inflammatory effect of exercise, both Bagby and colleagues (1994), and Kitamura and colleagues (2007), observed an exercise-induced suppression of plasma TNF-α after LPS administration. These systemic effects were seen following a single bout of exercise. Although an anti-inflammatory effect of exercise is possible, more research is warranted to determine whether anti-inflammatory effects of exercise are reproducible with exercise training.

6.6 Environmental enrichment and hippocampal function

It is important to appreciate that environmental enrichment may also have a positive effect on cognitive function. In a human population it is likely that environment is enriched by intellectual stimulation and social activities. The results from the chronic studies have allowed speculation that environmental

enrichment may have an effect on specific forms of hippocampal function in aged animals. In line with this, it is particularly pertinent that Lambert and colleagues (2005) suggest different elements of an enriched environment such as cognitive stimulation, exercise and acrobatic training have markedly distinct effects on spatial memory and synaptic alterations. Similarly, it has been proposed that different elements of an exercise program may have specific effects on hippocampal function.

Interestingly, an environmental enrichment effect was limited to aged animals in the present studies. Enhancements in learning and cellular modifications associated with learning were observed following regular handling and exposure to environmental stimuli over an 8-month period. There was no similar effect of environmental enrichment observed in middle-aged rodents. Harburger and colleagues (2007) investigated the age-dependent effects of environmental enrichment on spatial reference memory in male mice and demonstrated that 6 weeks of environmental enrichment significantly improved spatial memory in the MWM in aged males, but not in young or middle-aged groups. This result would appear to support a neuroprotective role for environmental enrichment. Additionally, Frick and colleagues (2003) suggest that environmental enrichment initiated at middle-age can reduce age-related impairments in spatial memory in mice. Escorihuela and colleagues (1995) also demonstrated that 6 months environmental enrichment, considered long-term environmental enrichment, could prevent the cognitive loss associated with aging. Similar to the effects of exercise, environmental enrichment has been associated with an enhancement in hippocampal neurogenesis in aged rats that has been linked with an improvement in spatial learning (Nilsson *et al.*, 1999). Despite this equivalent effect Olson and colleagues (2006) suggest that environmental enrichment and voluntary exercise should be considered distinct interventions with regard to hippocampal plasticity and associated behaviours. Where environmental enrichment increased the likelihood of survival of new cells, possibly by cortical restructuring, voluntary exercise increased the level of proliferation of progenitor cells. On the basis of these results, the authors proposed that the two manipulations affect different phases of the neurogenic process in different ways thus acting to enhance

neurogenesis via dissociable pathways.

But are the benefits to cognitive function more robustly produced with environmental enrichment or exercise? In the current experiments there is evidence that exercise and environmental enrichment may enhance some forms of hippocampal function. Published research has repeatedly shown that both interventions may enhance neurogenesis but it seems that more consistent neuroprotective effects are seen with exercise paradigms. Gobbo and O'Mara (2005) directly compared the effects of environmental enrichment and exercise on hippocampal degeneration induced by kainic acid and showed that exercise, but not environmental enrichment, improved learning and ameliorated the neurodegenerative decline associated with brain damage. Despite the suggestion that exercise may more reliably improve cognitive function, it is once again important to acknowledge that different elements of environmental enrichment, and different types of exercise, may have specific effects on the brain. It is possible that some forms of exercise may not improve cognitive function as effectively as environmental enrichment and vice versa.

6.6 Implications of research

This area of research is driven primarily from evidence of an expanding elderly population and their susceptibility to cognitive impairment, dementia and neurodegenerative disease. Having established that exercise can improve cognitive vitality in the elderly, research should now be focused on identifying the optimal exercising conditions for inferring this effect. Kramer and colleagues (2003), having reviewed both animal and human literature relating the effects of fitness training to cognitive function, stated that animal research clearly shows that exercise training can produce morphological and functional changes in the aged brain. Indeed, it is believed that exercise has the potential to confer neuroprotection in an aged population of animals and it is speculated that the exercise-induced effects on cognitive function are dependent on a number of factors. However, where the current research supports that of other animal studies, it is important to appreciate that further investigation concerning the types of learning improved by exercise is necessary. At present, the effects of exercise on

hippocampal-dependent learning have been well explored through animal research. However, the focus of human research has often been identifying the effects of exercise on executive functioning and frontal brain-dependent tasks. Recent work from this laboratory has shown that acute exercise can improve the performance of young healthy male human subjects in hippocampal-dependent tasks and that this is associated with increased BDNF in the circulation (Éadaoín Griffin, personal communication). Nevertheless, considering the necessity for animal research to be easily translatable to humans it is important to refine future animal studies so that the focus is more closely related to human function.

There is great promise shown by the wealth of human research that demonstrates the benefits of exercise training to learning and memory in elderly populations. Observational studies demonstrate more consistent support of the hypothesis that physical activity reduces the risk of cognitive decline in later life (Lautenschlager & Almeida, 2006). Although intervention studies that have been completed provide evidence of a causal relationship between exercise and cognition, there is a mixed pattern of results. In support of a neuroprotective effect of exercise Wang and colleagues (2006), investigating performance-based physical function and future dementia in the elderly, revealed that low levels of physical performance were associated with an increased risk of dementia and Alzheimer's disease. It has also been suggested that the exercise-induced effects on cognitive function extend to protecting against age-related brain atrophy. Colcombe and colleagues (2003) associated cardiovascular fitness with sparing of aged brain tissue. This result provides a strong biological basis for the role of aerobic fitness in maintaining and enhancing cognitive function in the elderly. A possible mechanism for this effect of exercise is enhanced CBV. Indeed, Rogers and colleagues (1990) associated physical activity in retirees with sustained cerebral perfusion and cognition.

To bring the focus back to the target population, the optimal exercise training required for improving cognitive vitality must be considered. Interestingly, Larson and colleagues (2006) reported that there was a greater risk reduction of dementia by exercise in cognitively intact older adults with lower levels of physical functioning, than with higher levels of physical functioning. In line with this, animal work completed by Kim and colleagues (2003) that has already been

mentioned, suggests that light-exercise produces more marked cell proliferation, a structural change that likely underlies an exercise-induced improvement in cognitive function. The impact of exercise to quality of life may also be relevant in regard optimum exercising conditions. Brown and colleagues (2004) investigated the associations between physical activity dose and health-related quality of life. The results showed that a better quality of life was associated with moderate levels of exercise compared with no exercise or longer duration and higher frequency of exercise. It has also been suggested that early life physical activity may delay late-life cognitive deficits (Dik *et al.*, 2003). Additionally, there is evidence that suggests women show more marked improvements in cognition with exercise, possibly by way of a synergistic relationship between BDNF and oestrogen (Berchtold & Kesslak, 2001). Given that a variety of factors may influence the extent to which exercise benefits cognition, it is clear that further research is warranted. Equally, it is important to acknowledge that an elderly population will presumably have a limited tolerance for exercise, specifically in relation to intensity and frequency, and perhaps efforts should be focused towards identifying the minimal amount of exercise that is required for inferring neuroprotection. Is it conceivable that a threshold of exercise for biological and neurological benefit exists?

In addition to exercise, a number of lifestyle factors may influence cognitive function in the elderly, such as intellectual engagement, social activities and diet (Kramer *et al.*, 2006). It is possible that these lifestyle factors are analogous to environmental enrichment interventions used in animal studies. Cognitive training has been shown to improve cognitive ability (Ball *et al.*, 2002). Furthermore, the effectiveness and durability of cognitive enhancement may be specific to the type of cognitive training undertaken (Willis *et al.*, 2006). There is also strong evidence that complex mental activity can improve clinical outcome from brain injury in the aged brain, possibly by way of neurotrophin induction (Valenzuela *et al.*, 2007). Considering dietary factors, it has been suggested that diet may powerfully influence brain function. Pinilla (2006) reported that a diet rich in saturated fat decreases learning and memory and increases metabolic distress, where diets supplemented with omega-3 fatty acids, vitamin E or curcumin (the

curry spice) benefit cognitive function. Indeed, Martin and colleagues (2002a) demonstrated that apoptotic changes within the aged brain that are associated with IL-1β signalling and impaired LTP, may be reversed by treatment with the polyunsaturated fatty acid eicosapentaenoic acid (EPA). Furthermore, EPA has been shown to produce a neuroprotective effect and reduce the vulnerability of the aged brain to amyloid-β (Lynch *et al.*, 2007). Interestingly, Pinilla (2006) proposed that the effect of exercise on cognitive function may overcome the negative effects of consuming a poor diet. Indeed, Wu and colleagues (2004) suggest that the interplay between oxidative stress and BDNF modulates the impact of a poor diet on synaptic plasticity and cognition, thus providing a possible mechanism by which exercise may override the effects of a poor diet.

6.7 Summary

This research has attempted to address whether the effects of exercise on hippocampal function are age-specific or exercise duration-specific. Indeed, the results suggest that the impact of exercise on cognitive function is exercise duration-specific, which is relevant when considering the optimal exercising conditions required for inferring neuroprotection. Importantly, it has also been shown that aged animals may equally benefit from exercise-induced improvements in cognitive functioning. It is especially encouraging that cognition and brain plasticity may be maintained in aged populations. In addition, the results lend support to the theory that an exercise-induced effect on the brain may be specific to types of hippocampal function. In light of this point, and previous discussion of existing gaps between animal and human research, it is of particular importance that future animal studies are more refined to allow clear translatability to humans. Despite these advances, the precise mechanisms by which exercise is mediating its effects cannot be fully elucidated. Although it is believed that BDNF is playing a role, the source of an up-regulation in BDNF is unknown and details of its precise mechanism of action remain unclear. Furthermore, it is speculated that an interplay of mechanisms, involving growth factors, angiogenesis and anti-inflammatory effects, may exist to generate the exercise-induced effects on cognitive function.

Finally, while it is appreciated that other interventions, such as mental acuity and dietary factors, may similarly provide neuroprotective effects, the elderly should be encouraged to participate in regular physical activity to preserve a healthy mind. To quote Marcus Tullius Cicero (~65 BC) "It is exercise and exercise alone that supports the spirits and keeps the mind in vigour."

6.8 Future Studies

This research has demonstrated that exercise has the potential to enhance cognitive function and confer neuroprotection that may ameliorate age-related impairments in hippocampal function. However, further research is warranted to more clearly define the mechanisms by which exercise mediates its effects on the brain. In addition, some unexpected observations open up interesting possibilities within this area of research and deserve more detailed investigation.

(1) BDNF-mediated mechanisms

Is BDNF directly associated with the exercise-induced effects on hippocampal function?

To identify a causal link between BDNF and exercise-induced effects on cognitive function the immunoadhesin (TrkB-IgG), that blocks TrkB ligands, could be used to block BDNF or BDNF knockout mice could be used.

If BDNF is playing a direct role what is the source of its up-regulation?

To identify if circulating BDNF is increased with exercise, concentrations of BDNF in the serum could be analysed. Isolation of neurons and glia would determine if BDNF is increased predominantly in either cell population. BDNF release experiments would provide further information about the source of an exercise-induced BDNF up-regulation.

Are other growth factors playing a significant role?

Are changes in the expression of IGF-1 in the periphery translated to the brain? Experiments could be completed to assess whether exercise up-regulates

circulating IGF-1. How is this effect associated with changes in BDNF expression?

What intracellular signalling pathways are activated by exercise?

Is TrkB activation altered by exercise? Is there a direct association between the activation of, for example, the PI-3K/Akt pathway to exercise-induced changes within the hippocampus. Is there a functional role for the transcription factor CREB?

(2) Exercise and neurogenesis

How significant are exercise-induced effects on neurogenesis in aged animals?

BrdU labelling studies could be completed to identify the impact of exercise-induced changes on neurogenesis in aged populations. Are the effects of exercise on neurogenesis in older animals exercise-specific or specific to some forms of hippocampal function?

(3) Exercise and vascular changes

How significant are exercise-induced vascular changes?

MRI could be used to identify exercise-induced effects on CBV and angiogenesis within different brain regions. Molecular experiments (a) could be used to identify changes within different brain regions in the expression of proteins associated with angiogenesis i.e. VEGF and angiopoietins and (b) could be used to determine whether exercise promotes an up-regulation of proteins associated with improved brain vascular integrity in the periphery. These changes could be correlated with exercise-induced improvements in cognition.

(4) Anti-inflammatory effects of exercise

Are the anti-inflammatory effects of exercise directly associated with exercise-induced improvements in cognition?

Are the anti-inflammatory effects of exercise that occur in the periphery translated to the brain? Further exploration of the effects of exercise on cytokines and inflammatory markers within the brain would provide valuable information with regard to the impact of exercise on age-related inflammatory change.

(5) Environmental enrichment

What elements of environmental enrichment are responsible for improving cognitive function?

Further investigation is required to clarify if the aged control animals used in the chronic exercise studies were subject to environmental enrichment by regular handling and exposure to environmental stimuli. Experiments could be completed to examine the impact of different elements of environmental enrichment on cognitive function. Are these effects age-dependent or duration-dependent? Do exercise and environmental enrichment have a similar magnitude of effect on brain function?

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VII Bibliography

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VIII Appendix

Solutions Used

Carbonate Coating Buffer pH 9.7

Sodium hydrogen carbonate	0.025M
Sodium carbonate	0.025M
Distilled water	

Electrode Running Buffer

Tris Base	25mM
Glycine	200mM
SDS	17mM

Krebs Solution

NaCl	136mM
KCl	2.5mM
KH_2PO_4	1.18mM
$MgSO_4$	1.18mM
$NaHCO_3$	16mM
Glucose	10mM
Containing $CaCl_2$	2mM

Phosphate-buffered Saline (PBS) pH 7.4

Na_2HPO_4	80mM
NaH_2PO_4	20mM
NaCl	100mM

PBS-Tween (PBS-T)

0.1% Tween-20 solution in PBS

Sample Buffer

Tris-HCL	0.5M, pH 6.8
Glycerol	10% v/v
SDS	0.05% w/v
B-mercaptoethanol	5% w/v
Bromophenol blue	0.05% w/v

Separating Gel

Bisacrylamide	33% w/v
Tris-HCL	1.5M, pH 8.8
SDS	1% w/v
Ammonium persulphate	0.5% w/v
Distilled water	
TEMED	0.1% w/v

Stacking Gel

Bisacrylamide	6.5% w/v
Tris-HCL	0.5M, pH 6.8
SDS	1% w/v
Ammonium persulphate	0.5% w/v
Distilled water	
TEMED	0.1% w/v

Transfer Buffer pH 8.3

Tris Base	25mM
Glycine	192mM
Methanol	20% v/v
SDS	0.05% w/v
Distilled water	

TBS-Tween Wash Buffer

Tris-HCL (pH 7.6)	20mM
NaCl	150mM
Tween-20	0.05% v/v
Distilled water	

IX Publications

Full Papers

O'Callaghan RM, Ohle R & Kelly ÁM. (2007). The effects of forced exercise on hippocampal plasticity in the rat: A comparison of LTP, spatial- and non-spatial learning. *Behav Brain Res* **176**, 362-6.

Hennigan A, O'Callaghan RM & Kelly ÁM. (2006). Neurotrophins and their receptors: roles in plasticity, neurodegeneration and neuroprotection. *Biochem Soc Trans* **35,** 424-7.

Minor Publications

O'Callaghan RM & Kelly ÁM. (2007). The effect of exercise on hippocampal function in young and aged male Wistar rats. *British Neuroscience Association Abstracts* **19,** 57.

O'Callaghan RM, Ohle R & Kelly ÁM. (2006). Exercise-induced changes in hippocampal function in the male Wistar rat. *Irish Journal of Medical Science* **175,** 128.

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