NOTE

Some images have been removed from the digital version of the thesis for copyright reasons.

Serotonin as a Mediator of Fatigue During Exercise and Training

by Daniel Dwyer (B.HSc.Hons.)

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

School of Health Science &
School of Physiotherapy and Exercise Science,
Griffith University, Australia.

June 2003

Abstract

Exercise has been shown to cause an increase in the concentration of brain serotonin (5-hydroxytryptamine, 5-HT) in humans and experimental animals. The increase in brain serotonin coincides with the onset of fatigue and is referred to as "central fatigue". Experiments in humans and animals involving serotonin receptor agonists have demonstrated reductions in exercise performance by simulating the exercise-induced increase in endogenous serotonin. Conversely, the administration of serotonin receptor antagonists has been shown to extend exercise performance in experimental animals, but not in humans. Although the relationship between the concentration of brain serotonin and exercise performance is well described in the literature, the precise effect of central fatigue on muscle function per se is unclear. Furthermore, there appear to be differences in serotonergic function between trained and untrained cohorts. However, it is not clear whether the differences are due to a training adaptation or if the differences are inherent in the individual. In addition, the time course of these adaptations and the mechanisms of adaptation are not known.

The initial purpose of this thesis was to determine whether six weeks of endurance exercise training had any effect on central serotonin receptor sensitivity in Wistar rats. The rats ran on a treadmill 4 times per week with 2 exercise tests of endurance performance per week. Receptor sensitivity was determined indirectly, at the end of each training week, by the reduction in endurance performance, under the influence of a 5-HT1a agonist, (m-Chlorophenylpiperazine, m-CPP). Improved tolerance to the fatiguing effects of the serotonin agonist would suggest desensitisation of central serotonin receptors, probably 5-HT1a receptors. Two groups of controls were used to examine, i) the effect of the injection per se on exercise performance and ii) changes in serotonin receptor sensitivity associated with maturation, in the

absence of any exercise training. In the training group, undrugged exercise performance significantly improved by 47% after 6 weeks of training (mean ± SEM, 4518 ± 729 s vs. 6640 ± 903 s, p=0.01). Drugged exercise performance also increased significantly from week 1 to week 6 (306 ± 69 s to 712 ± 192 s, p=0.004). Control group results indicated that the dose of m-CPP alone caused fatigue during exercise tests and that maturation was not responsible for any decrease in receptor sensitivity. Endurance training appears to stimulate an adaptive response to the fatiguing effects of increased brain serotonin, which may enhance endurance exercise performance.

The purpose of the second set of experiments described in this thesis was to investigate changes in serotonin receptor sensitivity in response to exercise training in human subjects. Twelve male volunteers completed 30 minutes of stationary cycling at 70% of VO_2peak, on 3 days per week, for 9 weeks. Serotonin receptor sensitivity was assessed indirectly by measuring the prolactin response to a serotonin receptor agonist (buspirone hydrochloride), using a placebo controlled, blind cross-over design. A sedentary group of control subjects were also recruited to control for possible seasonal variations in serotonin receptor sensitivity. Endurance capacity was also assessed as time to exhaustion while cycling at 60% of VO_2peak. The exercise training caused a significant increase in aerobic power (VO_2peak, 3.1±0.16 to 3.6±0.15 $L.m^{-1}$, $p< 0.05$) and endurance capacity (93±8 to 168±11 min, $p<0.05$), but there was no change ($p>0.05$) in the prolactin response to a serotonin agonist. However, 25% of the subjects in the training group demonstrated a decrease in receptor sensitivity, as indicated by a decrease in prolactin response. These results suggest that while the exercise training caused an increase in aerobic power and endurance capacity, there was no measurable change in 5-HT receptor sensitivity. In addition, it is possible that changes in receptor sensitivity may take longer to occur, the training stimulus used in the present investigation was

inadequate or that changes occurred in other 5-HT receptor subtypes that were not assessed by the present methodology.

The third set of experiments described here, investigated the changes in neuromuscular function under the influence of a serotonin receptor agonist (buspirone hydrochloride). Subjects were administered the agonist or a placebo in a blind cross over design. Measures of neuromuscular function included reaction time (RT), hand eye coordination (HEC), isometric neuromuscular control (INC), maximal voluntary isometric contractile force (MVIC-F), isometric muscular endurance capacity (IMEC) and various electromyographic (EMG) indices of fatigue in biceps brachii. A preliminary experiment was conducted to determine a drug dose that did not cause sedation of the research subjects. The agonist caused a significant ($p<0.05$) decrease in MVIC-F, INC and IMEC. There was a non significant ($p = 0.08$) decrease in EMG amplitude during the MVIC-F trial with the agonist, compared to the effect of the placebo. The median EMG frequency during the IMEC test was also significantly less with the agonist, when compared to the placebo effect. There was a decline in RT and HEC, although this was not significant. These findings indicate that a serotonin receptor agonist causes a decrease in neuromuscular function during isometric muscle contractions. The decrements in muscle function reported in this study may help to explain previous reports of an association between increased brain serotonin concentration and a reduction in endurance performance. Although the present study does not exclude the possibility that an increase in brain serotonin does cause fatigue by affecting organs peripheral to the brain, it provides evidence of fatigue within the central nervous system. Further examination of the effect of a serotonin agonist on muscle function during non-isometric muscle contractions is warranted.

Acknowledgments and Dedications

As a requirement for a subject in Exercise Biochemistry taught by Dr. Bon Gray, I had to write an essay on the performance limitations of a marathon race. During the literature search for this assignment, I opened the pages of “Endurance in Sport” by Shephard and Astrand. The description of central fatigue intrigued me then and obviously still does. Thank you Bon, for inadvertently showing me the way to the topic of my doctoral thesis.

I would like to acknowledge the academic support of my first supervisor and mentor, Dr. Jay Browning. Dr. Tony Perkins and Dr. Don Schneider have also contributed to my PhD and I thank them for their guidance. Thank you to Gary Dennis who provided valuable technical and scientific advice in the third experiment. In the second experiment, Dr. John Fynn performed the medical assessment of the subjects, Leahanna Baldock and Emma Green provided excellent phlebotomy services and James Pollock supervised a few of the training sessions. Other than these individuals, the conception and conduct of all experimental procedures was accomplished by the author himself.

Along my academic journey I have benefited from the influence of Dr. Bon Gray, Mr. Rod Barrett, Professor Greg Gass, Tim Gabbett, Clare Weber and Shaun D’Auria. The schools of Heath Science and Physiotherapy and Exercise Science have also provided me with the tangible requirements of my research.

Outside the university, my friends have allowed me to maintain balance and perspective in my life. I thank James Pollock, Sophie Gow, Greg Pooley, Mark Kulkens, Leigh Kalf, Simon White and Wiebke Warnken. My parents, sisters and their husbands have supported what I have done and I thank them for this.

I consider myself very fortunate to live in a community where I am able to work and study for years, without penalty to myself or anyone else. I have been able to view what is happening in the world and grasp some sense of my place in it. However, I often forget how fortunate I am and I take life's privileges for granted. I shall try not to do this in the future.

I dedicate this work to my parents, Brian and Shirley Dwyer. They are ultimately responsible for this thesis.

Statement of Originality

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

.

Daniel Dwyer

Publications Arising Directly from this Thesis

Dwyer, D. and Browning, J. (1999) Endurance training reduces sensitivity to a 5-HT1a agonist in rats. Journal of Science and Medicine in Sport. 2(4):428. (reviewed abstract)

Dwyer, D. and Browning, J. (2000). Endurance training in wistar rats decreases receptor sensitivity to a serotonin agonist. Acta Physiologica Scandinavica. 170(3); 211-216.

Dwyer, D. and Flynn, J. (2002). Short term aerobic exercise training in young males does not alter sensitivity to a central serotonin receptor agonist. Experimental Physiology. 87(1); 83-89.

Dwyer, D. and Dennis, G. (under review). The Fatiguing Effects of a Serotonin Receptor Agonist on Neuromuscular Function.

Publications Arising Indirectly from this Thesis

Rose'Meyer, R., Harrison, G., Dwyer, D., Garnham, B., Headrick., J and Willis, R. (2000) Altered purine metabolism in hearts from exercise trained rats. Journal of Cellular and Molecular Cardiology. 32(3):A8. (reviewed abstract)

Fenning, A., Harrison, GJ., Dwyer, D., Rose'Meyer, R., Brown, L. (2001) Cardiac adaptation to endurance exercise training in rats. Journal of Molecular and Cellular Cardiology. 23(6): A34.

Table of Contents

1.4 List of Figures

1.5 List of Tables

1.6 Abbreviations

5-HT	5-hydroxytryptamine (serotonin)
5-HIAA	5-hydroxyindoleacetic acid (metabolite of serotonin)
BCAAs	branch chain amino acids
C	Control group
Central	any site within the CNS
Central fatigue	fatigue in the CNS caused by an increase in serotonin
CNS	central nervous system
CSF	cerebrospinal fluid
DA	dopamine
DOPAC	dihydroxyphenylacetic acid (metabolite of DA)
EMG	electromyograph/electromyogram
FFAs	free fatty acids
HEC	hand eye coordination
INC	isometric neuromuscular control
IMEC	isometric muscle endurance capacity
m-CPP	m-chlorophenylpiperazine (5-HT1a agonist)
MAOI	monoamine oxidase inhibitor
MVC	maximal voluntary contraction
MVIC-F	maximal voluntary isometric contractile force
Peripheral	any site outside the CNS
Prl	prolactin
PNS	peripheral nervous system
RT	reaction time
SC	Sham control group
T	Training group
Ve	Ventilation rate ($L.min^{-1}$)
VCO_2	Rate of Carbon dioxide consumption ($L.min^{-1}$)
VO_2	Rate of Oxygen consumption ($L.min^{-1}$)

Aims

The purpose of this thesis is to identify any adaptations in the central serotonergic systems that occur as a result of exercise training and to describe the effect of a serotonin agonist on neuromuscular function. The experimental work in this thesis is divided into three sections, the first and second sections examine training induced changes in central serotonin receptor sensitivity in rats and humans and the third section describes the effect of artificially increasing brain serotonin on neuromuscular function, using a serotonin receptor agonist.

i) The aims of the first project were to measure any change in serotonin (1a) receptor sensitivity in rats during six weeks of exercise training, to describe the time course of this change and to determine whether receptor sensitivity is predictive of endurance capacity.

ii) The aims of the second project were to measure any changes in serotonin receptor sensitivity in humans after nine weeks of exercise training and to determine whether receptor sensitivity is predictive of endurance performance.

iii) The aim of the third project was to examine the effect of a partial serotonin agonist on five parameters of neuromuscular function: reaction time, hand eye coordination, maximal voluntary isometric contractile force, isometric neuromuscular coordination and isometric muscle endurance capacity.

Hypotheses

i) Six weeks of exercise training in rats will increase endurance capacity, increase tolerance to an agonist for serotonin (5-HT1a) receptors and the degree of tolerance will be predictive of endurance capacity.

ii) Nine weeks of exercise training in humans will increase aerobic power and endurance capacity and cause a decrease in the prolactin response to a partial serotonin agonist.

iii) The administration of a partial serotonin agonist, will lead to decreases in the following parameters of neuromuscular function; reaction time (RT), hand eye coordination (HEC), maximal voluntary isometric contractile force (MVIC-F), isometric neuromuscular coordination (INC) and isometric muscular endurance capacity (IMEC). Furthermore, spectral changes of the electromyogram during the MVIC-F and IMEC test will be indicative of exercise induced fatigue.

Significance

In the body of research available at the present time, more attention has been given to fatigue originating in organs outside the central nervous system (CNS) than the CNS itself. This is primarily due to the complexity of the CNS and its inability to be biopsied safely. As a result, far more is known about sites of fatigue that are peripheral to the nervous system and these areas continue to receive the focus of research. There is little doubt that there are several sites along the pathway of the production of movement that are responsible for fatigue, but their contribution to “total fatigue” and the nature of their genesis remain contentious (Kent-Braun, 1999). Nevertheless, the actual point of fatigue during physical work is likely to be determined by a combination of factors that are dependent upon the type of physical work being performed. These may include a combination of neural and biochemical factors. The remaining challenge is to describe the complete list of fatigue factors that limit performance and understand how they culminate in the onset of fatigue during exercise.

In the past, investigators have acknowledged that the CNS is a source of fatigue but have not described the precise mechanism of fatigue. Only recently has technology allowed the discovery of evidence that the nervous system is a source of fatigue and a limitation to work capacity (Bigland-Richtie et al., 1983). Prior to this, the role of the CNS as a determinant of work capacity was limited to the realm of psychology. Although this area has been developed extensively and is now a discipline in itself, it only provides a conceptual explanation for fatigue in the central nervous system.

Fatigue originating in the CNS is termed “central fatigue”. Central fatigue can also be divided into two distinct types, (i) fatigue that occurs in the acute phase (minutes) of

repeated muscle contractions and is likely to be due to a depletion of neurotransmitter or sub-optimal output of the motor areas of the CNS (Brasil-Neto et al., 1993), and (ii) fatigue that occurs after prolonged exercise which is due to the accumulation of a neurotransmitter that has inhibitory effects in the central nervous system. The present work deals exclusively with the second type of fatigue that occurs after prolonged exercise and is referred to as central fatigue throughout this thesis.

Preliminary research into central fatigue points to the possibility that the neurotransmitter serotonin is involved in the onset of central fatigue, although it is not the only candidate. Dopamine has also been implicated in central fatigue as it's concentration is partly regulated by serotonin and dopaminergic neurones that innervate descending motor neurones. Nevertheless, the role of serotonin continues to be explored due to some promising findings in the last two decades (Blomstrand et al., 1988).

The understanding of serotonin mediated fatigue remains incomplete at this time and leaves many unanswered questions. Animal studies have established that an increase in brain serotonin occurs as a result of exercise and that this increase coincides with the onset of fatigue and a reduction in work rate (Chaouloff et al., 1989). The effects of an increase in brain serotonin are well described and are consistent with the onset of fatigue (Davis and Bailey, 1997). There is no direct evidence of an increase in brain serotonin in humans because of technical limitations. However, humans demonstrate the same humeral changes seen in rats that bring about an increase in brain serotonin during prolonged exercise (Blomstrand et al., 1988). In addition, experiments that employ serotonin agonists to simulate central fatigue provide evidence of serotonin mediated fatigue in humans (Marvin et al., 1997; Wilson and Maughan, 1992).

What remains unknown is the nature of the adaptations of the serotonergic system to exercise training. Previous research has shown that training may cause an adaptive response by the serotonergic system that attenuates the effect of central fatigue in animals (Acworth et al., 1986) and humans (Jakeman et al., 1994). These adaptations include changes to the release of serotonin as well as changes in serotonin receptor expression and sensitivity. Changes to pre-synpatic receptor function may explain the changes in serotonin release (Dey et al., 1992), however the existence and consequences of changes in post synaptic receptor are largely unknown. Changes to receptor function may be an important adaptation that assists the explanation as to why training leads to improvements in exercise performance. Furthermore, an understanding the activity of the serotonergic system is important for broader health related issues. Previous authors have demonstrated altered serotonergic function is probably involved in disorders such as the over training syndrome, depression and chronic fatigue syndrome (Gastmann and Lehmann, 1998; Dey et al., 1992; Bakheit et al., 1992).

In addition, although an increase in brain serotonin is known to reduce running performance in humans (Wilson and Maughan, 1992) and rats (Bailey et al., 1993a), the mechanism/s of this fatigue have yet to be described. It is thought that an increase in the concentration of brain serotonin decreases neuromuscular function, but the findings of the only report of this effect were contradictory and inconclusive (Cunliffe et al., 1998). The decrease in exercise performance reported previously may be due to factors other than neuromuscular dysfunction, such as increased perceived exertion (Marvin et al., 1997) and decreased cardiac output (Bagdy et al., 1989). A better

understanding of this mechanism will provide a clearer picture of how central fatigue limits exercise performance and under what circumstances.

The present work provides evidence of a training induced adaptation in the serotonergic system in animals but not humans. The nature of the adaptation, the rate of onset and its implications for exercise performance are discussed. Furthermore, evidence that a serotonin receptor agonist causes a decrease in neuromuscular function is presented and its significance with respect to the findings of previous reports is discussed.

1.0 Review of the Literature

1.1 Introduction

The onset of fatigue during exercise may be an important evolutionary adaptation to prevent injury. Intense or prolonged exercise depletes energy stores, raises core temperature, dehydrates the tissues and can cause debilitating metabolic acidosis during intense work (Gollnick, 1982). These departures from homeostasis can cause tissue damage if allowed to persist and require a rest period for recovery. The processes that prevent physical work from becoming too intense or continuing on for too long, allowing adequate recovery might be referred to as fatigue mechanisms.

There are examples of marathon runners who have pushed through the experience of fatigue to the point of complete exhaustion and muscle damage in Commonwealth and Olympic games (Jim Peters, 1954 and Gabriella Anderson-Schiess, 1984). These individuals were able to “over exert” themselves despite intense feelings of fatigue and pain. The normal response to negative feedback, such as fatigue and pain associated with exercise, would usually be a decrease in intensity or cessation of exercise all together. The break down of this mechanism or the ability to over come it is likely to originate in the brain, where the neurological stimulus for locomotion originates and sensations of pain and fatigue are processed. In the previously mentioned cases, the athletes were apparently able to maintain the stimulus to exercise and ignore the strong sensations of pain and fatigue. Perhaps the most likely explanation for this is that the highest degrees of personal motivation can modify perceptions of pain and fatigue, although there is little empirical evidence to support this. Alternatively, it is also possible that fatigue may be attenuated or delayed during exercise, by adaptations in the brain that occur as a

result of exercise training. The present work explores this question and the more simple question of whether the capacity to perform exhaustive physical work (exercise), is limited by neural mechanisms in the CNS.

Competitive sport has generated a need for a greater understanding of the limitations to work capacity. Some of the earliest scientific work in this area occurred when Hill and Lupton (1923) measured the maximal rate of oxygen consumption in running human subjects. The last eighty years of research in exercise physiology has deepened our understanding of the limitations of exercise performance. This research effort has also incorporated other disciplines into new branches of exercise science such as biomechanics and exercise biochemistry. This thesis investigates the neural mechanisms of fatigue originating in the brain, as a limitation to exercise performance and examines evidence of an adaptation in the brain that delays this fatigue. The goal of this literature review is to summarise the mechanisms of fatigue, which are known to limit exercise performance. The literature review is primarily directed at mechanisms in the CNS that cause fatigue after a bout of endurance exercise (>50 minutes), given that this is most relevant to the experimental work in this thesis. The involvement of the CNS in exercise may be considered at two levels, cognitive and neurochemical. The neurochemical aspects of exercise induced fatigue are considered here and not the cognitive, as that is the domain of psychology. Other forms of neurochemical fatigue in the CNS and those peripheral to the CNS are summarised.

1.2 Fatigue as a Limitation to Exercise Performance

Fatigue as a limiting factor of exercise performance, has attracted much attention in the literature (Gollnick, 1982). The pursuit of improved exercise performance and an understanding of work physiology have lead to the exploration of the causes of fatigue in an effort to reduce or delay fatigue. The purpose of exercise training is to improve the functional capacity of the individual for competitive sport, recreation or health reasons. The improvement process occurs in two ways, by enhancing skill level or technique and by increasing physiological capacity to perform exercise. The maximum capacity of an individual to perform exercise is determined by a complex interaction of many biological processes (eg. glucose transport across the cell wall, calcium release into t-tubules). Each process has an important role and may by itself, fail or at least limit the exercise capacity of the individual. Thus, the capacity of an individual to perform exercise is probably determined by the point at which a single biological process reaches its maximal capacity. This point can be considered as the point at which the onset of fatigue occurs. The challenge for exercise physiologists is to identify precisely which biological process is the most important limiting factor. It is likely that there are many answers to this question as the there are many different forms of exercise that elicit different physiological responses.

Within the central nervous system, fatigue is likely to occur at one or more points in the sequence of processes that are responsible for muscular contraction. This sequence relies upon the motor cortex to provide the stimulus for contraction, the motor neurones to transmit the stimuli and the muscles to respond to the stimuli by contracting in a coordinated manner. As the components of this process are co-dependent, fatigue may occur when there is a decrement in function or a limit of activity has been reached in any of these components. In addition, the nervous system provides feedback from joint and muscle receptors to modify motor cortex

output, including inhibition, which offers a further mechanism for fatigue to occur. Fatigue may be defined as “an acute impairment of exercise performance that includes both an increase in the perceived effort necessary to exert a desired force or power output and the eventual inability to produce that force or power output” (Davis et al., 1992).

1.3 Central vs. Peripheral Fatigue

The majority of fatigue research has centred on peripheral sources of fatigue such as skeletal muscle and the circulatory system (Davis and Bailey, 1997). This is partly due to the fact that most tissue peripheral to the CNS can be sampled without any serious consequences, where as CNS tissue cannot. Additionally, the ability to introduce a treatment to control the function of an organ during exercise, thereby investigating its role, has not been possible in the CNS until recently. Only in the last few decades have pharmaceuticals been developed to assess the function of specific centres of the brain. There is now considerable understanding of the peripheral limitations to exercise performance and much of the current research continues on this theme (Baldwin, 2000).

1.4 Peripheral Factors of Fatigue

Sustained muscular contraction during exercise relies upon a complex physiological process that supplies fuel to myocytes and removes the metabolites of cellular respiration. The goal of this process is to provide adenosine tri-phosphate (ATP) for muscular contraction and prevent the accumulation of metabolites that may disturb cell homeostasis. ATP is required to release the myosin head from the troponin filament after contraction and then abduction of the myosin head in preparation for subsequent cross bridge attachment. The rate of ATP resynthesis within a muscle determines the maximum work rate of the muscle and hence exercise capacity. There are three metabolic pathways that provide ATP; the creatine phosphate system, anaerobic glycolysis and oxidative phosphorylation. The rate of ATP resynthesis may become insufficient for several reasons.

i. When the intermediate stores of creatine phosphate become exhausted,
ii. When there is an accumulation of metabolites (eg. hydrogen ions, H^+) that inhibits the activity of important enzymes (eg. phosphorylase, phosphofructo kinase)
iii. The demand for oxygen exceeds the rate of delivery and the rate of ATP resynthesis via oxidative phosphorylation, slows down.

1.4.1 ATP Resynthesis and the Energy Pathways

Creatine phosphate (CP) is a short term form of energy storage and is able to resynthesise ATP at the highest rate of the three pathways that are described here. This characteristic allows CP to be responsible for most of the energy supplied during intense bouts of work lasting up to ~12 s. Although CP stores are never fully

depleted and recover very quickly, the decrease in CP has been shown to coincide with fatigue (Saltin et al., 1972).

Anaerobic glycolysis provides limited ATP resynthesis during periods of intense exercise lasting for up to 3 minutes, when the energy stores of the CP system have been depleted. During periods of high demand for ATP resynthesis, the product of glycolysis, pyruvate, NADH and a H^+, form lactate and NAD^+. While this reaction does not directly produce energy for ATP resynthesis, it maintains glycolytic flux by ensuring a supply of NAD^+. NADH is normally converted to NAD^+, however during intense prolonged exercise, the demand for oxygen may exceed the rate of supply and there is an inadequate supply of NAD^+ for glycolysis. While anaerobic glycolysis can resynthesise ATP for a longer duration than the CP system, the by-product of anaerobic glycolysis is lactic acid, which dissociates its hydrogen ion to form lactate. When the work rate of exercise is sufficiently high, the rate of production of hydrogen ions may exceed the rate of removal and there is an inevitable decrease in pH within the muscle. The continued decrease in pH inhibits the activity of glycolytic enzymes (eg. phosphofructokinase) and hence the production of pyruvate (Spriet et al., 1987). The duration that ATP resynthesis can occur anaerobically, before the onset of fatigue, depends upon the intensity of exercise and the ability to buffer the increase in the concentration of hydrogen ions (Parkhouse and McKenzie, 1984).

Where work intensity is relatively low, the requirement for ATP resynthesis can be met aerobically via oxidative phosphorylation. Oxidative phosphorylation is the most efficient of the three energy systems and the duration of ATP resynthesis is determined by the total amount of available substrate if the work intensity is low enough. The Krebs cycle strips acetyl-CoA of its electrons, which then combine with oxygen to form water and the rephosphorylation of ADP to ATP, in the electron

transport chain. Under normal conditions, neither the rate of oxidation of substrate (glucose or fat) to form NADH, nor the rate of removal of metabolites (carbon dioxide) limits the production rate of ATP (reviewed by Bassett and Howley, 2000). Rather, it is the rate of oxygen delivery to the electron transport chain that limits the maximum rate of ATP resynthesis and NADH recycling. As this rate limit is exceeded, anaerobic glycolysis becomes responsible for more of the total ATP resynthesis. As most forms of exercise last for more than a few minutes, oxidative phosphorylation is the predominant form of ATP resynthesis and the most important of the three energy systems. Consequently, the maximal rate of oxygen consumption has become the standard measurement of work capacity and cardiopulmonary function.

During endurance type exercise, work rate is maintained within the capacity of oxidative phosphorylation, which allows sustained ATP resynthesis without the accumulation of hydrogen ions and subsequent fatigue. Therefore the factor that limits the duration of exercise before the onset of fatigue is the total amount of substrate available for the production of acetyl-CoA (Sjodin and Svedenhag, 1985). Acetyl-CoA is derived from glucose (stored as glycogen) via glycolysis or from triglycerides via beta oxidation. The production rate of acetyl-coA from glucose is higher than for triglycerides, therefore glucose tends to be the primary source of acetyl-coA during relatively intense exercise, while triglycerides are used at lower work rates (Sjodin and Svedenhag, 1985). Trained marathon runners store enough glycogen to sustain acetyl-CoA synthesis at a high rate for ~90 minutes (Sjodin and Svedenhag, 1985). Beyond this point, acetyl-CoA is produced from triglycerides and a small amount (~7%) from the catabolism of protein (Sjodin and Svedenhag, 1985). Although fat stores are vast and are not usually exhausted during endurance events, the production rate of acetyl-CoA from fat is slow which requires a decrease in work rate to avoid the accumulation of hydrogen ions and subsequent fatigue.

1.4.2 Cardiovascular Structure and Function

Muscular contraction relies upon the supply of ATP from three energy systems. Each energy system provides ATP resynthesis at different combinations of rate and duration. Fatigue occurs when; i) the ATP requirement exceeds supply, ii) there is an accumulation of metabolites that inhibit glycolysis, iii) when the rate of delivery of substrate is inadequate or iv) when the stores of substrate are exhausted. The delivery of substrate and removal of metabolites are critical components in two of the three energy systems. Therefore the importance of the cardiovascular system has been examined closely in the literature.

Oxygen travels in the blood stream primarily bound to haemoglobin and there is also a small amount in dissolved in plasma. Erythrocytes collect oxygen as they pass through the alveoli in the lungs, which amounts to ~200ml of oxygen gas, in each litre of blood. As the blood is pumped through capillaries, oxygen diffuses through the walls into the tissues along a concentration gradient. The rate of oxygen delivery is one determining factor of maximal aerobic capacity, which depends on the oxygen content of blood and the flow rate of blood through active muscle tissue.

The oxygen content of blood is determined by the rate at which oxygen diffuses across the alveolar wall and the capacity of the blood to carry oxygen. The oxygen diffusion rate across the alveolar wall is normally not a limiting factor to this process in healthy individuals when haemoglobin saturation in arterial blood is >98%. However, at very high work rates, some investigators have reported desaturation of arterialised blood (saturation of haemoglobin below 98%), which occurs when the oxygen diffusion rate is inadequate. The precise reason for this is not known but

current theories include pulmonary oedema and short transit time of erythrocytes through the lungs (Miyachi and Tabata, 1992). The ability of the blood to carry oxygen has been shown to effect performance (Jones and Tunstall-Pedoe, 1989). If the concentration of haemoglobin falls below optimal levels, the maximal rate of oxygen delivery is decreased. A decrease in haemoglobin concentration usually indicates iron anaemia to which athletes are at higher risk, than the sedentary population. The mechanisms of iron loss; sweat, foot strike haemolysis and gastrointestinal slapping occur more frequently in athletes (Balaban, 1992).

Unlike oxygen diffusion capacity in the lungs, cardiac output is a limiting factor of aerobic capacity in most individuals (Bassett and Howley, 2000). Increases in cardiac output are generally accompanied by increases in aerobic capacity (Spina, 1999). The presence of a pathology that reduces cardiac output also reduces aerobic capacity. Given normal regulation of blood flow during exercise away from non-essential organs (eg. kidneys) to the muscles, cardiac output determines the rate of oxygen delivery to the muscles. This also relies upon a network of capillaries throughout the muscle to facilitate the diffusion of oxygen into myocytes. As oxygen moves along a concentration gradient, the proximity of the cell to the capillary wall is a critical factor in determining the rate of oxygen delivery to myocytes. Higher capillary density in muscle tissue allows enhanced oxygen delivery by reducing the average distance between a myocyte and a capillary. Capillary density also influences the removal rate of metabolites such as carbon dioxide and hydrogen ions.

The delivery of glucose and fatty acids to working muscle is required for the production of acetyl-CoA. An inadequate supply of these fuels would reduce the production rate of acetyl-CoA and lead to a reduction in work rate or fatigue. However as described previously, there is ample storage of glucose (as glycogen)

in the liver and muscles and fatty acids stored as fat in adipocytes and in muscle. The rate of supply of these fuels is normally not a limiting factor to work capacity. However, when the supply of glucose is exhausted the continued oxidation of fatty acids requires a decrease in work rate.

1.4.3 Ventilation

Finally, adequate lung function is required to oxygenate venous blood and to exhaust carbon dioxide. In the absence of any pathology (asthma, chronic obstructive pulmonary disease) the rate of ventilation is not a limitation to the rate of oxygen delivery to the tissues or the rate of carbon dioxide removal. The possibility that ventilatory muscles (diaphragm and intercostals) become fatigued during exercise has been explored. Isolated mammalian diaphragm muscle does fatigue when electrically stimulated artificially (Roed, 1988). However, at the point of fatigue during a graded exercise test in human subjects, the peak ventilation rate is lower than the maximum voluntary ventilation rate (Bassett and Howley, 2000). This indicates that there are factors other than ventilation rate that are responsible for the cessation of exercise.

1.5 Control of Motor Function

The coordination of muscle contraction and movement is a complex process handled by many structures within the nervous system (Figure 1.1). A relatively simple movement, such as clapping, may involve thousands of synapses. The constituents of the motor control process can be divided into three main levels. The hierarchy of these levels in descending order of complexity is the motor cortex, the cerebellum and the brain stem-spinal cord. Each level or component acts on the level below, but does not replace its function. The volitional initiation of movement, or “fixed action movement patterns” begins in the motor cortex, but the actual planning of the movement occurs in the cerebellum and basal nuclei. The “signal” for movement is then communicated to the spinal cord and eventually to the target muscles. Some simple movements, or “reflex action movement patterns” such as the response to pain, are mediated by reflex arcs in the spinal cord and brain stem (Grillner and Wallen, 1985)

The lowest level of the motor control hierarchy consists of segmental circuits that innervate a collection of motoneurones for specific groups of muscle fibres. These spinal circuits control movement and are called central pattern generators (CPG). CPGs are characterised by their ability to produce a rhythmic discharge of action potentials independently of input from the higher level components of the motor control system. The rhythmic discharge from CPGs excites motoneurones in a pattern that produces common types of movement (eg. walking). This rhythmic movement can be seen in neonates and animals with spinal damage as they rely on CPGs for movement (Wallis, 1994). Although CPGs can work independently in the intact nervous system, the activity of the CPGs is controlled by higher order neurones that form part of the cerebellum and motor cortex. Initiation of movement can occur in response to activity in the brain stem, chemical activation in the spinal

cord or to sensory stimuli. Within the spinal cord, agonists of muscarinic, dopaminergic and serotonergic receptors can initiate movement (Cazalets et al., 1992). Supra-spinal influence over the CPGs is probably mediated by serotonin and noradrenaline (Jacobs and Fornal, 1999).

The spinal cord, and hence, the CPGs are under the direct influence of a large array of neurones that project from the motor cortex and the cerebellum. These neurones help to regulate reflex and fixed action movement patterns. So called “command neurones”, modulate the activity of CPGs and are themselves influenced by sensory input. Pyramidal neurones of the cerebral cortex also innervate the spinal cord and are thought to be responsible for fine voluntary movements. The most numerous projection neurones are the reticular, vestibular, red nuclei and superior colliculi systems (review by MacKay-Lyons, 2002). These systems are involved in the integration of ascending sensory input with subsequent descending commands and are responsible for the majority of typical involuntary (eg. posture, eye movement) and voluntary (eg. standing up, opening a door) movements. Primary afferents from sensory receptors provide information about muscle tension (golgi tendon organs), rate of change of muscle length (bag chain fibres), joint angle (proprioceptors) and pain (nociceptors) to the central pattern generator, the projection neurones and the cerebellum. Regulation of opposing muscles also occurs by modulating reflex inputs and motoneurone output (Grillner and Wallen, 1985).

Above the level of the projection neurones are the “pre-command” areas of the brain the cerebellum and motor cortex. The cerebellum is the primary site where the integration of sensory information and descending commands occur. The cerebellum is responsible for precisely starting and stopping movements, coordinating movements with posture, modulating muscle tone and blocking unwanted movements (MacKay-Lyons, 2002).

While the decision to initiate a movement occurs in the cerebral cortex the pre-command areas of the brain are inactive, until the “signal” is transmitted. Once this occurs, the pre-command areas are activated to plan the movement and the motor cortex is inactive (Latash, 1998). Once the movement occurs, both the cerebellum and the motor cortex actively process sensory input and refine descending commands. It seems that the pre-command areas provide the “order” to move and the motor cortex actually executes the movement.

Figure 1.1 The Structures Involved in the Generation of Movement Patterns (Marieb, 2001)

1.6 Central Factors of Fatigue

Despite the lack of empirical evidence, most researchers in the area of exercise physiology would acknowledge that the CNS is a determining factor of maximum work capacity. Indeed, "psychological factors" have been known to affect exercise performance for a century (Asmussen, 1979; MacLaren et al., 1989). CNS fatigue or central fatigue is sometimes suggested as a default explanation when peripheral factors do not account for total fatigue (Yue and Cole, 1992). However, more recently at least one report provides good evidence of central fatigue during exercise (Lepers et al., 2002).

Sports psychology recognises the importance of the mind/brain for exercise performance and attempts to exploit this to improve performance. However psychology relies upon the cognitive and behavioural realm and does not offer a direct biochemical explanation for central fatigue. Prior to the late 1980s the absence of a reasonable biochemical hypothesis for central fatigue hindered research in this area. As a result, the influence of the CNS on fatigue during exercise is less well understood than the peripheral factors of fatigue described previously. An added hindrance to this type of research is that the CNS is difficult to analyse, as the human brain cannot be biopsied for such purposes. Recently the development of nuclear magnetic spectroscopy and positron emission tomography has provided an opportunity to analyse the brain biochemistry in the intact human. To date there are only a few studies that have measured serotonergic activity in the intact human brain.

The CNS is responsible for many processes involved in locomotion and offers several possibilities for explaining centrally mediated fatigue during exercise. These processes exist on two levels in the CNS, the higher brain centres that are

responsible for cognition where the motivation to work is developed and at the motoneuronal level, where the motor cortex provides the neural drive for muscular contraction. Either level may mediate the onset of fatigue independently or in concert. On the cognitive level, work capacity is partly determined by motivation, which might be characterised by the relationship between the experience of fatigue and the willingness to endure the fatigue. At the neuronal level, muscle contraction requires optimal output from the motor cortex, uninhibited transmission of the signal through the CNS and peripheral nervous system (PNS) to the neuromuscular junction (see Figure 1.2). The author acknowledges that psychology may be an important the factor in this process, but confines the present work to the importance of neural function.

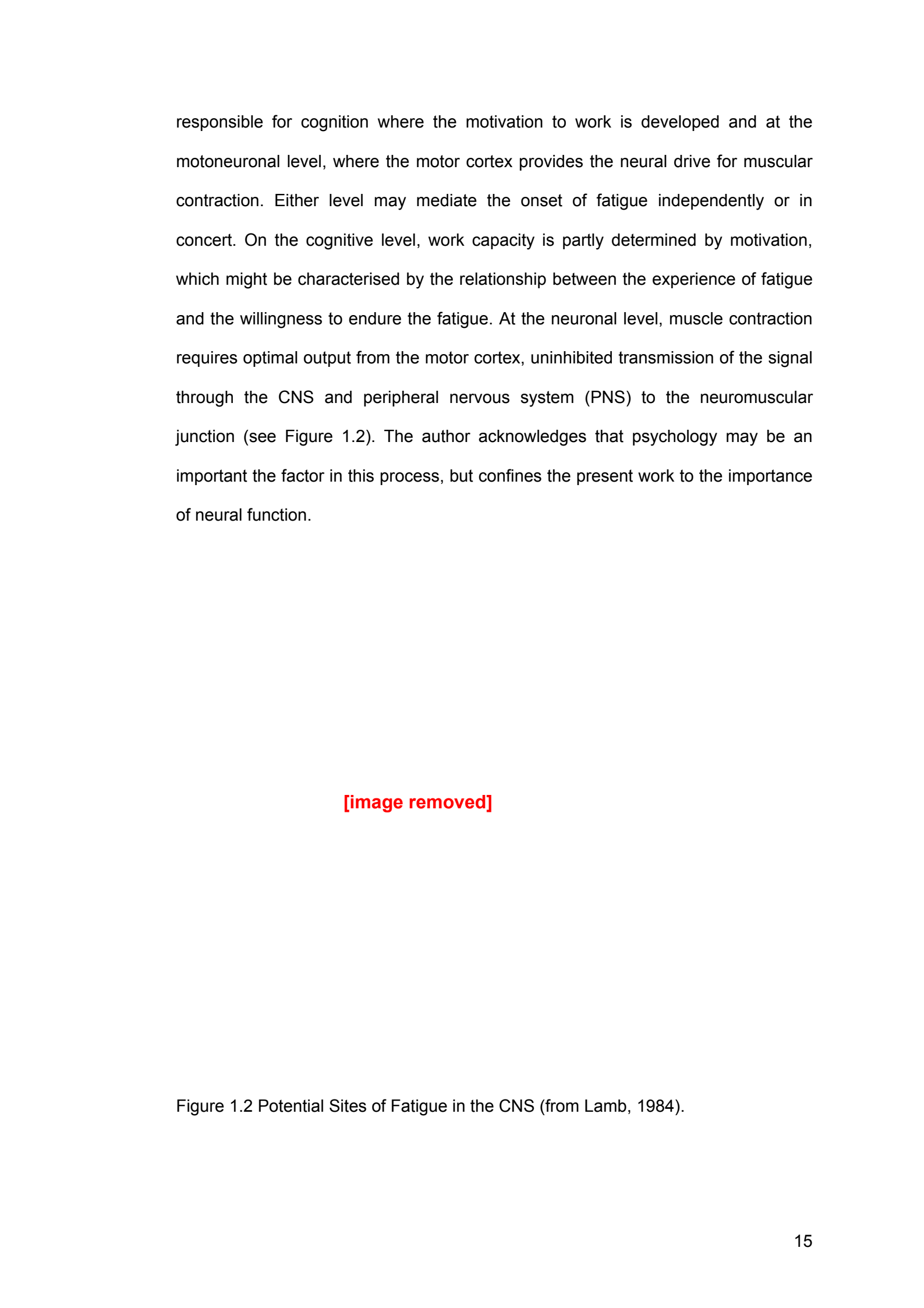


Figure 1.2 Potential Sites of Fatigue in the CNS (from Lamb, 1984).

For the purposes of this discussion, fatigue in the nervous system will be divided into two categories each characterised by the period over which fatigue develops. The following section reviews the evidence for fatigue in the nervous system that occurs within minutes. This type of fatigue develops within a series of contractions and may recover quite quickly. The subsequent section (1.7) is more relevant to this thesis as it describes fatigue that occurs after continuous exercise lasting more than forty minutes originating in the CNS. Both types of fatigue are limitations to exercise performance. However, as they develop over different periods of time, they contribute differently to the total experience of fatigue during exercise.

1.6.1 Neurological Fatigue During Acute Bouts of Exercise

There is an opportunity for any of the biological components of the nervous system involved in muscle activation to become defective and cause fatigue. The action potentials generated in the motor cortex must be transmitted, unhindered, to the motor end plate and then be propagated throughout the motor unit. Any decrement in the performance of the pathways involved in this process could reduce the number and or frequency of motor units recruited during a muscle contraction. Additionally, the afferent signals from the working muscle that mediate the neural drive may also be involved in the onset of neural fatigue.

1.6.2 The Central Nervous System

Evidence that the CNS is involved in fatigue has been gathered using several different experimental approaches. Mental activity has been shown to delay fatigue during repeated isometric contractions. Subjects in one study completed mental

arithmetic during each trail and were able to improve their endurance by 46% (Kotz et al., 1978). An investigation into the effect of mental stress on the contraction endurance of the middle finger of one hand revealed a decrease of 45% (Asmussen, 1979). Further work in this area reveals that during both concentric (Asmussen, 1979) and isometric (Secher, 1987) muscle contractions, work is decreased when the eyes are kept closed. These authors suggested that fatigue is enhanced when a subject concentrates on fatigue and although they did not provide a plausible mechanism to explain this, the same study also indicated that diversionary thoughts could reduce the perception of fatigue (Secher, 1987). In this last example it is assumed that the activity in the higher brain centres interferes with the signals responsible for muscular contraction. Furthermore diversionary thoughts appear to speed recovery after fatigue. Mental activity or opening the eyes at the point of fatigue (where the eyes where shut during exercise) have been shown to reduce recovery time (Secher, 1987).

The enormous complexity of the interaction between thought and motor functioning means that it is presently not possible to reach firm conclusions. Most of the evidence that supports the importance of the role of the CNS on fatigue comes from studies of the action potentials that drive muscle contraction. More specifically, the evidence comes from studies employing, twitch interpolation and transcranial magnetic stimulation (TMS) techniques.

The most common research model used to delineate fatigue that occurs in the periphery from that occurring in the CNS, involves the stimulation of motoneurones whilst the subject performs a maximum voluntary contraction (MVC) (Taylor and Gandevia, 2001). The stimulation can be achieved by applying an impulse current to the skin above the motoneurone (twitch interpolation) or via an electromagnetic pulse directly into the motor cortex (using TMS). The experimental design assumes

that during a prolonged MVC, when there is a decrease in force associated with fatigue, if stimulation of the motoneurone increases force, then the muscle is not truly fatigued. The suggested mechanism for this increase in force is that the voluntary effort is not recruiting all of the motor units or they are not firing fast enough and the stimulation of the motoneurone redresses this deficit. The implication of this evidence is that part of the decrease in contractile force during prolonged or repeated contractions is due to sub-optimal output of the motor cortex and not local fatigue mechanisms in the muscle alone. During an MVC, this may account for 10-20% of force loss and up to 50% during other fatiguing protocols (Lloyd et al., 1991).

The use of TMS to investigate motor cortex excitability has provided further evidence to support a central origin of fatigue during exercise. The application of an electromagnetic pulse to the motor cortex elicits a short excitatory response called a motor evoked potential (MEP) that descends the corticospinal neurones and can be seen in an electromyogram of the target muscle. The MEP typically consists of several waves (a D wave and several I waves) that reflect the stimulus pattern of the motor cortex and descending motoneurone. The appearance of the MEP on an EMG during a voluntary contraction is followed by a period of electrical silence. The length of the silent period reflects inhibition of motoneuronal drive, reduced excitability of motoneurones and intracortical inhibition (Taylor and Gandevia, 2001).

Motor cortex excitability can be assessed in terms of the size (or amplitude) of the MEP and the length of the silent period. Figure 1.3 illustrates typical changes in the amplitude of the MEP after a bout of fatiguing exercise. The decrease in amplitude (at 4 minutes post exercise) indicates decreased excitability of the motor cortex (Samii et al., 1996). Further work by Taylor and Gandevia (2001) has shown an increase in the duration of the silent period during a sustained MVC that is

associated with fatigue. Taken together, these findings lend support to the notion that during acute bouts of exercise, the CNS demonstrates evidence of fatigue. Additional work is required to fully characterise the nature of this type of fatigue and its effects on subsequent exercise performance.

Figure 1.3 Post-exercise facilitation and depression of Motor Evoked Potentials (MEPS). MEPs were evoked by trains of 5 transcranial magnetic stimuli delivered at 0.3 Hz. Exercise consisted of 3 x 30s contractions at 50% of maximal force and caused facilitation. Further bouts of 30s contracts occurred until fatigue, which caused depression of MEPs (from Samii et al., 1996).

1.6.3 The Spinal Cord

There is evidence that in some circumstances, a reduction in the EMG signal may be caused by increase reflex inhibition occurring in the spinal cord. This inhibition occurs in the α-motoneurone pool by afferent fibres originating in the working muscle. Changes in the spinal reflexes associated with stretch have been reported in fatigued muscles, particularly the monosynaptic reflexes (Avela and Komi, 1998).

The precise mechanism of the inhibition is unknown, however, decreased muscle spindle firing rate has been implicated (Avela et al., 2001). Alternatively the nerve endings of group III and IV afferent fibres may be responding to an increase in the concentration of muscle metabolites associated with a prolonged contraction (Avela et al., 2001). Bigland-Ritchie et al. (1986b) found that after a fatiguing MVC, mean motoneuronal discharge rate was depressed. Furthermore there was no recovery in discharge rate while the muscle was kept ischaemic, but recovered after three minutes of normal blood flow. Their conclusion was that motoneuronal discharge rate was regulated by a peripheral spinal reflex that responded to the fatigued state of the muscle.

1.6.4 The Final Motoneurone

Fatigue of the motoneurone in neuromuscular transmission was eliminated by some of the earliest studies in neurophysiology. When a motoneurone is artificially stimulated at “physiological” frequencies, there is no apparent decline in the fidelity of the signal conducted. The eventual fatigue of the neurone occurs much later than the decrease in contractile force, which provides evidence that the motoneurone is probably not responsible for neuromuscular fatigue (review by Simonsen, 1971).

1.6.5 The Neuromuscular Junction

Electrical stimulation of the final motoneurone has shown that the motor-end plate may be a site of fatigue. Stephens and Taylor (1973) recorded a decrease in EMG activity during both voluntary and artificial activation of the muscle. This finding suggests that the neuromuscular junction can fail because the EMG signal should

not have diminished during artificial activation. A further decline in force that was more rapid than the decline in EMG was noted in the voluntary activation of the muscle. This probably reflects an alteration in the recruitment pattern of the motor units. The more fatigable fast twitch fibres may be more susceptible to fatigue in the motor end plate while the slow twitch fibres may submit to fatigue in the muscle itself. Fatigue in the motor-end plate is thought to be due to the diminished release of acetylcholine in the axon or diminished release of calcium from the sarcoplasmic reticulum (Lamb, 1984).

1.6.6 The Muscle

Metabolic fatigue within the muscle has been discussed previously, however, there is another mechanism by which fatigue can occur. For an entire motor unit to function properly, action potentials that arrive at the motor end plate must propagate throughout the motor unit. This process relies upon optimal functioning of the sarcoplasmic reticulum and the T-tubules. There is evidence of slowing of the conduction velocity in the muscle, an increase in the excitation threshold of muscle fibres, and slowing of the relaxation phase post contraction (Latash, 1998). All of these changes have been shown to occur after a prolonged isometric contraction and would be manifest as fatigue. Although similar fatigue characteristics would be present during repetitive concentric type exercise, it is unclear how critical these changes are for work capacity involving complex patterns of muscle recruitment.

1.7 Neurological Fatigue During Chronic Bouts of Exercise

Although a distinction is made between the acute fatigue mechanisms summarised in the previous section and the chronic mechanisms discussed in the following section, the former are likely to play an important role in the "total" fatigue experienced during exhaustive exercise. The experience of fatigue is a complex phenomenon that is difficult to study, as it is often a combination of the effects of fatigue from diverse origins. In the following section the role of dopamine in the onset of fatigue in the CNS will be discussed briefly and the role of serotonin more thoroughly. There is evidence that the neurotransmitter, acetylcholine and neuromodulators such as ammonia and cytokines may be involved in the onset of fatigue. However presently, the majority of the evidence supports the involvement of serotonin and dopamine (Davis and Bailey, 1997).

1.7.1 The Role of Dopamine

Dopamine (DA) has been implicated in the onset of CNS fatigue because of it's important role in locomotion (Davis and Bailey, 1997) and because of the observation that the use of amphetamine improves exercise performance in animals (Bhagat and Wheeler, 1973) and humans (Gill et al., 2000). Additionally, apomorphine and L-dopa are dopamine receptor agonists that have been shown to affect locomotion in animals (Tamasy et al., 1981). In general, it appears that the metabolism of DA increases during exercise and decreases after exercise, which is associated with fatigue (Chaouloff et al., 1986a; Speciale et al., 1986). The changes in DA metabolism in rats are localised to regions in the brain including the midbrain, hippocampus, corpus striatum and the limbic system (Elam et al., 1987). Bailey et al. (1993a) examined changes in brain serotonin, DA and its metabolite (dihydroxyphenylacetic acid, DOPAC) in rats during exercise. The brain

concentration of DA was found to increase during exercise and then decrease during fatigue, which might lead to a decrease in the activation of motoneurones and a subsequent decrease in neural drive. In addition, these authors found that when brain DA synthesis was maintained, fatigue was delayed.

The precise mechanisms of the effect of DA metabolism on fatigue are unclear, although there appears to be an inverse relationship between the concentration of serotonin and DA during exercise (Chaouloff et al., 1987, Chaouloff et al., 1989). Indeed Naoi et al. (1994) found that DA inhibits tryptophan hydroxylase activity which may explain the relation between the two, and some of the fatigue effects mediated by serotonin. The relationship between serotonin and dopamine suggests that functionally, they are closely linked. Whilst there has not been a mechanism of fatigue identified that is primarily mediated by dopamine, serotonin has been directly implicated and may exert its influence by causing changes in the concentration of dopamine.

1.7.2 The Role of Serotonin

Serotonin was isolated from serum in 1948 by Page who was looking for a factor in the circulatory system that was responsible for vasomotor tone. The local effects of serotonin on the walls of blood vessels were quickly characterised (Rapport and Green, 1948) and its molecular structure described (Rapport et al., 1948). In 1954, Woolley and Shaw found that vasoconstriction is only a minor activity of serotonin and that it was much more important as a neurotransmitter in the CNS.

The majority of serotonin exists in chromaffin cells that control gut motility. Serotonin is also stored in and released by platelets at the site of tissue damage as

a chemotactic agent (Struder and Weicker, 2001). Of the total amount of serotonin in the body, only 1-2% is found in the CNS.

Despite the wide availability of serotonin from dietary sources, it is synthesised entirely from the amino acid tryptophan. Foods rich in tryptophan include pineapples, bananas and nuts. Tryptophan is absorbed through the intestinal mucosa and the liver enzyme, tryptophan pyrrolase, regulates its circulating concentration via degradation by the kynurenine pathway.

Tryptophan exists in two forms; bound and unbound to albumin. The unbound form accounts for ~10% of total tryptophan in circulation in resting subjects. Only the unbound or “free” tryptophan can cross the blood brain barrier, which occurs via an active transport mechanism (L-system transporter, Fernstrom and Wurtman, 1971). Although a more recent report suggests that the binding of tryptophan to albumin may not affect its ability to cross the blood brain barrier (Fernstrom and Fernstrom, 1993). The L-system transporter is also responsible for the entry of other large neural amino acids into the brain, such as the BCAAs (leucine, isoleucine and valine). Because tryptophan and the BCAAs compete for entry into the brain via the same mechanism, the rate of entry of tryptophan into the brain is governed by the ratio of free tryptophan to BCAAs (Pardridge, 1977). The concentration of testosterone and cortisol affects the binding affinity of albumin for tryptophan, which also affects the concentration of free tryptophan and hence the rate of entry of tryptophan into the brain (Struder and Weicker, 2001).

Once in the CNS, tryptophan is taken up by a facilitated transport mechanism into serotonergic neurones. The first step in this process is the hydroxylation of tryptophan at the 5 position by tryptophan hyroxylase, yielding 5-hydroxytryptophan

(5-HTP). 5-HTP is then decarboxylated by a non-specific amino acid decarboxylase enzyme to form 5-hydroxytryptamine (5-HT).

Figure 1.4 The Synthesis and Degradation of Serotonin (Rang, Dale and Ritter, 1999)

It is generally thought that any increase in brain tryptophan leads to an increase in brain serotonin, since the enzyme tryptophan hydroxylase, is usually only half saturated (Hamon et al., 1981). Serotonin is stored in secretory vesicles that become attached to the presynaptic membrane within the axon body, from where it

is released into the synaptic cleft (Rudnick and Clark, 1993). Once released into the synaptic cleft and then from its receptor, it is either taken up by the axon again for re-release or it is metabolised into 5-hydroxyindoleacetic acid (5-HIAA). This metabolite is found in cerebrospinal fluid and in urine and may reflect qualitative rates of turnover of serotonin (Rang, Dale and Ritter, 1999). The release of serotonin is attenuated by the stimulation of presynaptic 5-HT1a,b and d autoreceptors (Barnes and Sharp, 1999).

1.7.3 Architecture of Central Serotonergic Neurones

Serotonergic neurones extend into nearly all parts of the brain and are involved in the regulation of a wide variety of functions. The development of techniques to map neural networks in the CNS (autoradiography, fluorescence histochemistry and nuclear magnetic resonance imaging) and probe their function with selective ant/agonists drugs has allowed the gradual discovery of the nature and role of serotonergic neurones. Modern pharmacology has made use of this information to develop treatments for eating disorders, migraine, depression and anxiety. The role of serotonin in the regulation of sleep and locomotion has also been explored and is closely related to the present work.

Despite the billions of cells in the CNS, serotonergic cells only number in the thousands. Although they comprise a small portion of the total, they exhibit greater influence than their numbers suggest. It is estimated that there are ~$6x10^6$ serotonergic varicosities / mm^3 of cortical tissue in the rat brain. By extrapolation serotonergic terminals may account for 1/500 of all axon terminals in the cortex of the rat (Audet et al., 1989).

In the CNS serotonergic neurones are found in the brain stem and are divided into two main groups; superior and inferior. The superior group is comprised of four main nuclei and the inferior group contains five nuclei (see Table 1.1).

Superior Group	Inferior Group
Caudal linear nucleus	Nucleus raphe obscurus
Median raphe nucleus	Nucleus raphe pallidus
Lateral neurones positioned dorsal to the medial lemniscus	Nucleus raphe magnus
Dorsal raphe nucleus	The ventrolateral medulla
	Intermediate reticular nuclei

Table 1.1 Serotonergic Neurones in the Central Nervous System

[image removed]

Figure 1.5 The Distribution of Serotonergic Neurones in the Brain (Rang, Dale and Ritter, 1999). Str: striatum, Sep: septum, Hyp: hyopthalamus, Th: thalamus, SN: substantia nigra, Hip: hippocampus, Am: amygdala and C:cerebellum.

1.7.4 The Function of Serotonergic Neurones

Serotonergic neurones are involved in many functions that are not related to regulation of motor function, such as appetite, mood and thermoregulation (Bobker and Williams, 1990). However the present discussion is restricted to the involvement of the serotonergic system in motor functions.

Serotonergic neurones exhibit a range of activity levels that vary with the sleep-wake arousal cycle. This includes inactivity during REM sleep through to 4-7 spikes.s^{-1} during waking state (Jacobs and Fornal, 1997). The strong relationship between arousal and activity suggests the primary role of serotonergic neurones in the CNS is to send information to target neurones regarding the level of motor activity and behavioural state. The slow and regular rate of spikes suggests that the information is simple and probably regulatory. Although the degree of influence on target neurones would also depend on the density of innervation of serotonergic cells (Jacobs and Fornal, 1997). Aghajanain and Vandermaelen (1986) concluded that serotonergic neurones might act to facilitate motor systems while suppressing sensory systems. Therefore during arousal, motor systems are facilitated and sensory systems are suppressed, conversely when the organism is drowsy or sleeps motor systems are inhibited and sensory input is facilitated. The serotonergic system may also regulate the output of the autonomic nervous system to match the level of motor activity.

The functions of serotonin are not all regulated by the same type of receptors. The identification and characterisation of these receptors has been an ongoing process that has seen the classification of some receptor types change. The most recent classification system recognises fifteen different receptors (Barnes and Sharpe,

1999). The following table summarises the distribution and effects of each receptor subtype in the central nervous system.

Receptor	Type	Distribution	Behavioural Function
1A	G protein linked	Hippocampus, lateral Septum, Cortex	Locomotion, Thermoregulation, Appetite
1B		Basal Ganglia, Striatum	Locomotion, Appetite
1D		Basal Ganglia, Hippocampus, Cortex	Not Characterised
1E		Cortex, Amygdala, Hypothalamus	Not Characterised
1F		Hippocampus, Cortex, Dorsal Raphe Nucleus	Locomotion, Thermoregulation
2A		Throughout the Forebrain, Hippocampus	Thermoregulation
2B		Cerebellum, Lateral Septum, Hypothalamus, Amygdala	Mood
2C		Choriod Plexus, Cortex, Limbic System, Basal Ganglia	Locomotion, Appetite, Mood, Sexual Function, Thermoregulation
3	Gated ion channel	Brain Stem, Hippocampus, amygdala	Locomotion, Appetite, Mood, Sexual Function
4		Nigrostriatal, Mesolimbic Regions	Mood, Cognition, Locomotion
5A	G protein linked	Not Well Characterised	Mood, Cognition
5B		Not Well Characterised	Not Characterised
6		Striatum, Hippocampus, Cortex	Not Well Characterised
7		Thalamus, Hypothalamus, Hippocampus	Not known

Table 1.2 Serotonin Receptor Subtypes in the Central Nervous System. (Adapted from Barnes and Sharpe, 1999, Also see Jacobs and Fornal 1993 for the basic properties of serotonin neurones).

1.7.5 The Involvement of Serotonergic Neurones in Locomotion

The importance of serotonergic neurones for locomotion could be based upon the distribution of serotonergic neurones in the CNS alone, because the brain stem and α-motoneurones receive input from a dense collection of serotonergic neurones (see section 1.7.3). However, the initial links between serotonergic function and motor activity were made in studies that artificially increased the concentration of brain serotonin. The resulting hyperactivation of motor centers caused a variety of symptoms including, hyperactivity, “wet dog shakes”, tremor and fore paw treading in rats (Jacobs, 1976). More recently a similar response under the influence of high doses of serotonin agonists has been demonstrated in humans (Sternbach, 1991). The effects of injecting serotonin directly into specific motor areas of the CNS on motor function have also been shown to increased muscular activity during walking (Barbeau and Rossignol, 1991) and increased EMG activity (Ribiero-do-Valle, et al., 1991).

The bulk of research into the role of serotonergic neurones in the control of movement has been made on the cat. These studies have focussed on two main brain areas; the forebrain (pontine-mesenphalic raphe nuclei dorsalis) and the brain stem-spinal cord (Medullary raphe nuclei magnus, obscurus and pallidus). The activity of these regions parallels the sleep-wake-arousal cycle. Serotonergic neurones in the dorsal raphe nuclei, the largest collection of serotonergic neurones in the CNS, discharge at about 3 spikes.s^{-1} (Jacobs, 1991) during quiet awake state, through to electrical silence during REM sleep. As REM sleep is characterised by muscle inhibition, the relationship between neuronal activity and locomotion has been examined carefully. Injection of a serotonin receptor agonist (5-H1a) into the dorsal raphe nuclei (DRN) induces REM sleep (Monti et al., 2000). Experimental

animals are able to track visual stimuli and can be considered conscious, however activity of the DRN was non-existent and the animal is paralysed (Jacobs and Fornal, 1997).

[image removed]

Figure 1.6 Activity of a Serotonin Neurone in the Dorsal Raphe Neucleus of the Cat. REM, rapid eye movement. (from Jacobs and Fornal, 1993).

Studies on isolated spinal cord and brain stem have also demonstrated the role of serotonergic function in locomotion. During activities mediated by the CPG such as chewing, licking, stepping, there is an increase in neuronal activity of 50-100% (Veasey et al., 1995). Veasey et al. also demonstrated a positive correlation between the magnitude of neuronal activation and the frequency of the activity (ie. speed of walking or breathing). Work by Kiehn and Kjaerulff (1996) has also shown that serotonin applied to neonatal rat spinal cord causes fictive locomotion.

Thus, it appears that in the cat, there is a strong relationship between the activity of serotonergic neurones in the DRN and movement, which is supported by other work

(Fornal et al., 1996). However, there is large variability in this relationship between brain areas, which may reflect their degree of involvement in the control of locomotion. For example, the increase in activity of serotonergic neurones in the caudal medulla is less than half that of the DRN, during locomotion. Jacobs and Fornal (1997) conclude that, “serotonergic neurones play an auxiliary role in coordinating appropriate autonomic and neuroendocrine outputs to the ongoing tonic or repetitive motor activity”.

Serotonin has also been shown to act directly on 5-HT1 and 5-HT2 receptors on motoneurones (Cazalets, et al., 1992). It appears that the effect of serotonin on motoneurones only occurs when outflow in the neurone is intact. The effect is a facilitation of neuronal activity, which is mediated by 5-HT2a and 5-HT2c receptors (Wite et al., 1996). Analysis of intracellular mechanisms indicates that this is probably due to depolarization achieved by reducing the membrane conductance to potassium. It appears that serotonin shifts motoneurones from a hyperpolarised state with little activity to a depolarised state with steady neuronal activity. Indeed the nature of serotonin neurones is to discharge at a steady rhythmic rate (Jacobs, 1991).

The prevalence of serotonergic neurones throughout the CNS has made it’s relationship with motor control relatively easy to establish. However, it’s complexity also makes it difficult to reveal the details of the facilitatory and inhibitory functions of the serotonergic system. A critical element of this relationship is the fact that the effect of serotonergic activity on locomotion exists on a continuum. Some serotonergic activity is associated with normal locomotion and very high activity results in the serotonin syndrome (ie. motor dysfunction). Fatigue during prolonged exercise is associated with serotonergic activity somewhere between these two points (Davis and Bailey, 1997).

The role of serotonergic neurones in the control of movement has been established from numerous animal studies (Jacobs and Fornal, 1997). While there is a paucity of similar human studies, the animal models (rodent and feline) of the human motor system appear to have been useful surrogates, because the response of the motor system to perturbation of the serotonergic system is common across the vertebrates (Jacobs and Fornal, 1993). However, the fact that the human motor system has not been directly investigated in great detail remains a limitation of the current understanding of this topic.

1.7.6 The Inhibitory Characteristics of Serotonergic Neurones

There is now a large body of evidence demonstrating that the main post synaptic action of serotonin is inhibition, although there is evidence of excitation as well, (review by Jacobs and Azmitia, 1992). The bulk of this research has identified an inhibitory action of serotonergic neurones in the forebrain, specifically implicating the inhibitory characteristics of the 5-HT1a receptor in the dorsal hippocampus and neocortex. Additional investigations into the actions of serotonergic neurones in the spinal cord reveal inhibition of somatosensory neurones (Aghajanian and Vandermaelen, 1986). Inhibition by the 5-HT1a receptor is brought about by hyperpolarisation of the target neurone by increasing conductance to potassium without dependence on calcium (Segal, 1980). Conversely, the 5-HT2 receptor appears to reduce the degree of inhibition of the 5-HT1a receptor (Davies et al., 1987). The postsynaptic inhibition by serotonergic neurones appears to rely upon normal activity of the target neurone. If serotonin is applied to the somatosensory cortex of anaesthetized rats there is little or no effect on these neurones. When serotonin is applied in conjunction with an excitatory stimulus to the cells, their

activity is suppressed (El-Yassir et al., 1988). Similar findings have been reported by Spampinato et al. (1985) who examined the effect of the serotonin agonist, m-CPP, on dopamine synthesis in the striatum of rats. Inhibition only occurred when the impluse flow of dopaminergic neurones was intact. This finding is supported by further work that showed a decrease in activity of dopaminergic neurones in the striatum of rats after spontaneous running was used to stimulate an increase in brain serotonin (Elam et al., 1987).

Serotonin also directly inhibits the action of serotonergic neurones themselves. Auto inhibition appears to be modulated by the 5-HT1a receptor in the somatodendritic regions of the neurone (Aghajanian and Vandermaelen, 1986). Inhibition is achieved in the same way as postsynaptic control, by increasing the conductance to potassium into the neurone (Aghajanian and Lakoski, 1984).

Amongst the research that examines the activity of serotonergic neurones *in vitro* and collections of neurones *in vivo*, there are a couple of published reports on their inhibitory effect on descending motoneurones (Spampinato et al., 1985; Akai et al., 1991) and at least one that supports an excitatory effect (Roberts and Wright, 1981). It is difficult to clearly identify an overall inhibitory effect of serotonergic neurones on descending motoneurones for several reasons. The complexity of the motor system means that the presence of serotonergic neurones with inhibitory characteristics in a motor area cannot by itself serve as evidence that serotonergic neurones inhibit motoneurones. The presence of excitatory neurones elsewhere in the motoneurone pathway may mitigate these inhibitory effects and a large portion of motoneurones may not be influenced by inhibitory neurones. In addition, many of the studies that investigate the inhibitory characteristics of serotonergic neurones were performed before antagonists were available for specific receptor subtypes. The classification system for serotonergic neurones has changed twice since 1986,

which also confuses the interpretation of receptor function studies prior to this period.

1.8 The Effect of Exercise on Serotonergic Function

A neurohumoral hypothesis to explain the mechanism of fatigue and sleep was first presented by Pieron (1912). Pieron's hypothesis did not explain the biochemistry involved in the regulation of sleep, but suggested that changes in the circulating levels of hormones lead to changes in the CNS that caused fatigue and eventually sleep. This hypothesis was supported by later work (Schwenk, 1965). Further investigations identified the increase of a factor in the brain after exercise that caused inhibitory effects on CNS function (Romanowski and Janota-Lukaszewska, 1967). The same study demonstrated that when extracts of brain tissue containing this factor were taken from animals exhausted by exercise, it was found to cause fatigue when it was introduced into the CNS of non-exercised animals. A similar experiment has been reported recently, where cerebrospinal fluid from exhausted mice was shown to cause fatigue when injected into the brain of rested mice (Inoue et al., 1998). Subsequent research (from 1967) implicated serotonin as the most likely factor, due to its sedative effects in the CNS (Romanowski and Grabiec, 1974). However, Blomstrand et al. (1988) were the first authors to present a mechanism to explain the exercise induced increase in brain serotonin and hence, the complete central fatigue hypothesis.

1.8.1 Serotonin Synthesis

The rate of synthesis of brain serotonin depends upon the rate of entry of tryptophan into the brain, which is dependant upon the ratio of "free" tryptophan and

the branch chain amino acids (BCAAs; Pardridge, 1977). Therefore, an increase in brain serotonin can be achieved by increasing the concentration of free tryptophan or decreasing the concentration of the BCAAs.

At rest about 80-90% of total tryptophan is bound to albumin (McMenamy, 1965). Plasma Free Fatty acids (FFA) also bind to albumin and have a greater affinity for albumin than does tryptophan (McMenamy, 1965). The onset of exercise stimulates lipolysis, which increases the concentration of FFAs, which displaces tryptophan from albumin, increasing the concentration of "free" tryptophan (Curzon et al., 1973). This effect alone would lead to an increase in the ratio of tryptophan to BCAAs and the subsequent increase in the rate of entry into the brain. However, this ratio is also affected by a change in the concentration of amino acids during exercise.

Circulating concentrations of the BCAAs generally decreases during exercise, as they are taken up by active muscle as a minor source of fuel. There are discrepancies that exist in the literature on this matter and they are likely to be due to differences in the intensity and duration of exercise. The prevailing view is that exercise of low intensity and long duration is more likely to cause a decrease in the concentration of amino acids (Struder and Weiker, 2001). Work in this area has demonstrated an increase in the ratio of tryptophan to BCAAs after exercise in animals (Blomstrand et al., 1989) and humans (Struder et al., 1996).

However one recent study casts doubt on this contention. Work by Nybo et al., (2003) examined the effect of 60 minutes of cycling on the rate of entry of BCAAs and free tryptophan in to the brain. The rate of entry was estimated by the arterio-venous difference in the concentration of BCAAs and free tryptophan. They reported a no significant change in the rate of entry of tryptophan into the brain. The authors

concede that 60 minutes of exercise may not have been long enough to have any effect.

Therefore the increase in the ratio of free tryptophan to BCAAs during exercise probably leads to an increase in the rate of entry of tryptophan into the brain. The processes that synthesise serotonin from tryptophan are not considered to limit the rate of production of serotonin, which include enzyme activity (Newsholme and Leech, 1983), transport of tryptophan across blood brain barrier (Wurtman, 1983) and transport across the cell membrane of the neurone (Young, 1986). In addition, depolarisation of serotonergic neurones leads to an increase in the activity of tryptophan hydroxylase and hence, an increase in the rate serotonin synthesis, where there is an adequate supply of tryptophan (Chaouloff, 1997).

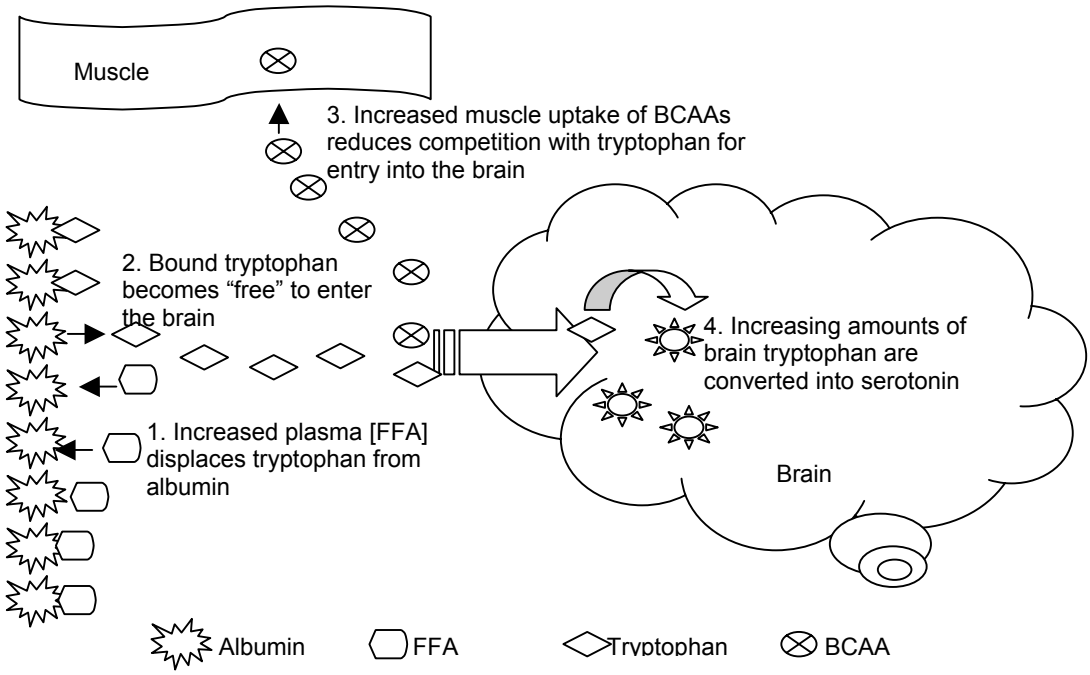


Figure 1.7 The Effect of Exercise on the Synthesis of Serotonin. 1. Exercise stimulates an increase in plasma FFAs. 2. Tryptophan is displaced from albumin by FFAs. 3. BCAAs are taken up by muscle and allow entry of tryptophan into the brain. 4. Increased entry of tryptophan into the brain causes an increase in the synthesis of serotonin.

An additional factor that may increase the concentration of brain serotonin is the apparent increase in blood brain barrier permeability during exercise. A novel experiment using Evans blue dye bound to albumin reported increased entry of the protein-dye complex, suggesting leakage of large molecules through the blood brain barrier during exercise (Sharma et al., 1986). While the study didn't measure the rate of entry of tryptophan into the brain, it is likely to have increased given that it is a much smaller molecule than albumin (66,200 vs. 204 Daltons). Although if this were the case, one might expect to see an increase in all varieties of amino acids in the brain after exercise, which was not the case in at least one other report that examined the phenomenon in rats (Chaouloff et al., 1986a).

In summary, there are two important humoral changes during exercise that lead to an increase in the rate of entry of tryptophan into the brain; i) the increase in the concentration of FFAs that displaces tryptophan from albumin and increases the concentration of free tryptophan and ii) the decrease in the plasma concentration of BCAAs, which decreases competition for entry into the brain via the transporter molecule (Blomstrand et al., 1988).

1.8.2 Evidence for Serotonin Mediated Fatigue

1.8.2.1 Animal Studies

The effect of exercise on serotonin synthesis has been examined by measuring the concentration of tryptophan, serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in samples of brain tissue and cerebrospinal fluid, pre and post exercise. The primary goals of research thus far, have been to establish the serotonergic response to exercise in terms of the increased rate of entry of tryptophan into the

brain and an increase in the production and release of serotonin and subsequent degradation.

It has been assumed that a rise in brain tryptophan would lead to an increase in the production of serotonin, which will facilitate the inhibitory and fatiguing effect of serotonin during exercise. This assumption is based on the fact that the enzyme that synthesises serotonin is only normally half saturated (Hamon et al., 1981) and an increase in brain serotonin causes fatigue and decreased locomotion. Short term exercise has been shown to increase whole brain tryptophan in several studies (Chaouloff et al., 1985, 1986a, 1986b, Acworth et al., 1986). A detailed study by Blomstrand et al. (1989) reported increases in tryptophan in brain regions that had some involvement in locomotion (cerebellum, hypothalamus, hippocampus, brain stem, striatum). While an increase in tryptophan suggests increased entry into the brain, these studies measured the concentration of extracellular tryptophan, which is related to it's rate of release but does not directly indicate it's intracellular concentration.

There are also reports of an increase in brain serotonin, post exercise (Acworth et al., 1986, Meeusen et al., 1996). The study of brain regions by Blomstrand et al. (1989) found increased serotonin in the hypothalamus and non-significant increases in the striatum, brain stem and the hippocampus, but not the cerebellum or cortex. Chaouloff et al. (1985) and Heyes et al. (1988) found non significant increases in the whole brain and the striatum, brain stem and hypothalamus.

A useful technique that allows real time measurement of changes in brain neurochemistry during exercise involves the implantation of a micro dialysis probe. The use of this technique has been reported several times, finding an increase in serotonin in the brain stem after exercise in rats (Gerin et al., 1994), and an

increase in the hippocampus (Wilson and Marsden, 1996). A more recent report found an increase in tryptophan and serotonin in the hippocampus (Gomez-Merino, et al., 2001). These results suggest that a significant increase in serotonin occurs only in localised areas of the brain. This probably reflects the function of these brain regions and their involvement in locomotion. The areas that appear to reliably demonstrate increases in serotonin during exercise include the striatum, hippocampus and hypothalamus.

A post exercise increase in the metabolite serotonin (5-HIAA), would indicate an increase in the turnover of serotonin that presumably would only occur where there is increased production of serotonin, given normal or depressed concentrations of serotonin. There are several reports of an increase in whole brain 5-HIAA concentrations, post exercise (Dey, et al., 1992, Gerin et al., 1994, Chaouloff et al., 1985). There are also reports of significant increases in the striatum (Heyes et al., 1988, Blomstrand et al., 1989) and the hippocampus (Blomstrand et al., 1989, Gomez-Merino et al., 2001). Conversely Meeusen et al. (1996) found a non-significant increase in the hippocampus and Acworth et al. (1986) found no increase in any brain region, although the trained rats in this study did demonstrate an increase in 5-HIAA. Chaouloff et al. (1986b) reported an increase in the concentration of 5-HIAA in cerebrospinal fluid, however this could only be considered to be a qualitative indicator of changes in brain 5-HIAA.

Taken together, these results present a consistent serotonergic response to exercise, although it appears that the response is specific to certain brain regions. There is an increase in the concentration of tryptophan and serotonin in response to exercise and this lasts for up to 90 minutes after the cessation of exercise (Gomez-Merino et al., 2001). The interpretation of this response is problematic given that this response may reflect changes in the rate of degradation of both tryptophan and

serotonin. The reports of changes in the concentration of 5-HIAA during or post exercise are perhaps the best indicator of the rate of turnover of serotonin. An increase in brain 5-HIAA most likely occurs because of an increase in serotonin production and degradation. An alternate explanation of this is that it may be due to an increase in the degradation of serotonin without any increase in production of serotonin. However, where an increase in the concentration of 5-HIAA has been reported, serotonin concentration has remained the same or increased (Chalouff et al., 1985, Blomstrand et al., 1989, Gerin et al., 1994, Meeusen et al., 1996, Heyes et al., 1988, Gomez-Merino et al., 2001). There are no reports of an increase in 5-HIAA and a decrease in serotonin, which lends support to the conclusion that exercise increases the rate of turnover of brain serotonin in rats.

1.8.2.2 Human Studies

There are no reports that provide direct evidence of an increase in brain serotonin during exercise in human subjects. The major problem with investigations of this type is the technical difficulties involved in measuring brain neurochemistry. Direct sampling of brain tissue in humans is not possible and concentrations of metabolites in cerebrospinal fluid or urine indicate only qualitative changes in serotonergic activity. Therefore there are only a limited number of reports of the changes in plasma amino acids in response to exercise in human subjects. Specifically, investigators have sought to determine whether there is an increase in the free tryptophan to branch chain amino acid ratio and then infer that this is likely to lead to an increase in brain serotonin.

Blomstrand et al. (1988) examined changes in plasma amino acid concentrations after two types of exercise; a marathon and an army training program lasting ~1.5

hours. Both types of exercise caused a decrease in the concentration of plasma BCAAs and an increase in plasma free fatty acids. The free tryptophan to branch chain amino acid ratio was significantly increased after the marathon and was not measured in the army training group. Similar responses have been reported in response to an ultra marathon (Lehmann et al., 1995), one hour of cycling (Fischer et al., 1991), 90 min of cycling (Paul et al., 1996), cycling to fatigue (Davis et al. (1992) and 5 hours of cycling (Struder et al., 1999). The work by Fischer et al. (1991) identified a increase in serum prolactin, concurrent with exercise, which was taken as an indicator of increased serotonergic activity in the brain. Increased serotonergic activity stimulates the hypothalamic-pituitary axis and stimulates the release of prolactin. This relationship has been widely used as an indirect measure of serotonin release and/or receptor function (Bridge et al., 2001).

No further work has been completed in this area and definite conclusions about the existence of an increase in brain serotonin, made on the basis of these results alone are problematic. However, if the evidence from animal models is taken into account, it is reasonable to contend that, exercise leads to an increase in brain serotonin in human subjects. Further support for this contention is offered from drug studies that are discussed later. Another limitation of research in this area is that nothing is known about the temporal response of serotonin during exercise, or how work intensity affects the changes in brain serotonin. These features will remain poorly understood until technology allows direct measurement of the neurochemistry in the intact human brain.

1.8.2.3 Nutritional Supplement Studies

The identification of a mechanism of fatigue often results in attempts to control or delay fatigue for performance enhancement or the treatment of a relevant pathology. There are several stages along the serotonin synthesis pathway where it might be possible to prevent its increase in the brain during exercise and the subsequent fatigue effects. The most obvious stage to intervene is the entry of tryptophan into the brain. It is normal for tryptophan to enter the brain, but the increased rate of entry during exercise is abnormal and if prevented would presumably have little or no effect on homeostasis. The mechanism of increase relies on an increase in plasma free fatty acids and a decrease in BCAAs. Attenuating the increase in plasma FFAs might prevent the increased rate of entry of tryptophan into the brain, but it may also limit the availability of fats as an energy substrate. It is the second option that offers a strategy for preventing the increased rate of entry of tryptophan into the brain without disturbing the supply of substrate for muscle metabolism. In addition, experiments that are successful in reducing the entry of tryptophan into the brain that also show an increase in performance (presumably via an attenuation of the increase in brain serotonin), would provide supporting evidence for the central fatigue hypothesis.

Work by Blomstrand et al. (1991a) showed that supplementation of BCAAs to slower marathon runners (3:05-3:30 hr) but not faster marathon runners (<3:05 hr) significantly improved performance. Supplementation has also been shown to be responsible for a two fold increase in endurance performance in rats (Yamamoto and Newshome, 2000; Calders et al., 1999). Yamamoto and Newshome (2000) found that administration of BCAAs resulted in a decrease in the uptake of tryptophan into striatal neurones by 22%. Alternatively, branch chain amino acid supplementation has been reported to have no effect in humans (Varneir et al., 1994, Paul et al., 1996) and a detrimental effect in exercising rats (Verger et al., 1994). Van Hall et al. (1995) compared the effects of tryptophan and two different

doses of BCAAs on cycling performance. They found no change in cycling endurance capacity despite being able to demonstrate that the treatments increased and decreased the plasma concentration of free tryptophan. The failure of branch chain amino acid supplementation to enhance performance may be due to increased plasma ammonia concentrations, which can be toxic to the brain (MacLean and Graham, 1993). In addition, the ergogenic effect of BCAA administration may be confounded by their ability to increase insulin release which would reduce carbohydrate availability during exercise (Davis et al., 1992).

The contradictory nature of these results is difficult reconcile, however some of the differences may be due to experimental factors. Varnier et al. (1994) found no increase in performance after supplementation but used subjects in a glycogen depleted state. Therefore, peripheral factors of fatigue such as substrate depletion may have been more critical in determining performance, than central fatigue. Additionally, the results presented by Verger et al. (1994) indicating a decrease in performance after supplementation, may be explained by an increase in insulin. More well controlled studies of the effect of BCAAs on exercise performance in man are required to confirm the efficacy of this strategy. The ergogenic effect of BCAAs can only be confirmed when an optimal dose is identified that doesn't stimulate an increase in insulin or ammonia and still inhibits the entry of tryptophan into the brain.

An alternate hypothesis that appeared briefly in the literature was that tryptophan supplementation would offer enhancements to exercise performance by improving tolerance to the painful effects of exercise. Serotonin is involved in nociception in the CNS and an increase in serotonin is thought to act as an analgesic (Akill and Liebeskind 1975). Segura and Ventura (1988) administered L-tryptophan to athletes and reported an increase in running performance by ~50%. However, the trials were an average duration of 5 minutes and would not have been long enough to allow the

full metabolism of tryptophan. The improvement in performance was most likely due to enhanced familiarity with the test protocol. The work was repeated by Stensrud et al. (1992) who found no positive effect of tryptophan supplementation on running performance.

Tryptophan supplementation has been used to support the central fatigue hypothesis by facilitating an increase in brain serotonin and causing fatigue during exercise. Cunliffe et al. (1998) found that the administration of tryptophan caused an increase in the perception of fatigue and a decrease in reaction time. However they also failed to detect a decrease in grip strength and found a paradoxical increase in wrist ergometric work. Tryptophan supplementation in horses has been shown to reduce performance in line with the expected effect of increased brain serotonin and the early onset of central fatigue (Farris et al., 1998).

1.8.2.4 Pharmacological Studies

Some of the most compelling data to support the central fatigue hypothesis involves the administration of serotonin antagonists, agonists and selective serotonin reuptake inhibitors (SSRIs). Where a serotonin antagonist extends exercise performance in a particular exercise test, it may be surmised that central fatigue is the limiting factor. The administration of selective serotonin agonists or antagonists allows the identification of the receptor subtype/s involved in central fatigue and the nature of central fatigue (ie. neuromuscular and/or psychological effects) by facilitating or blocking central fatigue.

Serotonin agonists have been shown to reduce performance in exercising rats (Bailey et al., 1992, 1993a, 1993b). These authors used 5-HT1a and 5-HT1c selective agonists to cause fatigue in exercising rats.

[image removed]

Figure 1.8 The Effect of a Serotonin Agonist on Running Performance in Rats (Bailey et al., 1992). M-chlorophenylpiperazine (m-CPP) is a serotonin (5-HT1a) agonist. * indicates a significant difference ($p < 0.05$) from vehicle, # indicates a significant difference ($p < 0.05$) from 1 $mg.Kg^{-1}$.

Dose response curves also indicate the sensitivity of the receptors and the range over which they are active. Bailey et al. (1992) demonstrated a nearly linear relationship between dose and exercise performance for a 5-HT1a agonist in exercising rats (Figure 1.8), which was also demonstrated for another agonist in a subsequent study (Bailey et al., 1993b). Although it is difficult to compare drug doses with levels of endogenous serotonin, this data lends further support to the notion that increased brain serotonin leads to a reduction in exercise capacity.

The blockade of serotonergic receptors during exercise has been shown to delay fatigue and extend exercise performance in rats using a dual 5-HT1c and 5-HT2

antagonist (LY 53,857; Bailey et al., 1993a, 1993b). These authors reported an increase in run time of up to 50%, although the dose-response was not linear (Figure 1.9).

Figure 1.9 Effect of a Serotonin Antagonist on Running Performance in Rats (Bailey et al., 1993b). LY 53,857 is a serotonin (5-HT1c,2) antagonist. * significantly greater run time compared to vehicle.

Ahlenius et al. (1997) examined the effect of a 5-HT1a agonist (8-OH-DPAT) and reported a biphasic effect on exercise performance. There was an initial increase in treadmill performance at low doses followed by a decrease in performance at higher doses. The initial increase in performance probably indicates the affinity of the agonist (8-OH-DPAT) for presynaptic receptors. Activation of these autoreceptors would inhibit the release of endogenous serotonin and hence the activation of post synaptic receptors. A further increase in the dose of the agonist was accompanied by a reduction in performance that may be accounted for by the activation of post synaptic receptors. These results indicate that the relationship between the

concentration of serotonin and the net effect of central fatigue is complex and that the poor selectivity of research drugs sometimes confounds results.

There are very few reports of the effects of drugs that affect serotonergic function in human subjects during exercise. The few available support the role of serotonin in the onset of fatigue during exercise. Marvin et al. (1997) examined the effect of the partial serotonin agonist buspirone, on short duration endurance performance on a bicycle ergometer. They reported a significant decrease in performance and an increase perceived exertion, compared to the effect of a placebo. Wilson and Maughan, (1992), examined the effect of a selective serotonin reuptake inhibitor (paroxetine), on cycling performance and also found a significant decrease in performance. Although Meeusen et al. (2001) performed a similar study but found that there was no effect of a different selective serotonin reuptake inhibitor (fluoxetine) on cycling performance, these authors conceded that their exercise protocol and drug dose may explain why they failed to detect any effect of fluoxetine.

There are only two reports of attempts to extend exercise performance in humans with a serotonin antagonist (Pannier et al., 1995; Meeusen et al., 1997). Although antagonists have been shown to enhance performance in rats, both reports using human subjects demonstrated a non significant decrease in run time to fatigue. Pannier et al. (1995) conceded experimental errors such as the selectivity, dose and side effects of the drug as possible explanations for the failure to improve performance. While Meeusen et al. (1997) questioned the validity of the central fatigue hypothesis entirely. These authors also reported a decreased catecholamine response to exercise under the influence of the antagonist, which may partly explain the decrease in performance. On the basis of previous work examining the response of rats to antagonists, it is likely that an efficacious dose of a 5-HT1a

antagonist may reduce central fatigue and increase performance. Although, the side effects of the antagonist must be minimal and benefit will only occur when the duration of exercise is long enough for central fatigue to be a determinant of performance.

Evidence from the animal experiments could be used to strongly support the central fatigue hypothesis, given that both antagonists and agonists have been shown to improve and reduce exercise performance. The evidence from human studies is less supportive, in that there is only evidence of decreased performance under the influence of serotonin agonists. An important complicating factor in most drug studies is the effects of the drug on serotonin receptors that are not involved in locomotion. For example, some serotonin agonists have been shown to reduce heart rate and blood pressure (Bagdy et al., 1989). This would undoubtedly compromise any conclusions about the precise origin of fatigue during an endurance exercise test. Similarly, the effect of an agonist on exercise performance may be explained by the adverse effects of a high dose (eg. sedation), rather than direct inhibition of descending motoneurones (Pannier et al., 1995). A useful alternate strategy would be to prevent the increased rate of entry of tryptophan into the brain, which has been reported by Yamamoto and Newsholme, (2000). These authors used a drug (aminobicycloheptane carboxylic acid) to inhibit the large amino acid transporter on the blood brain barrier in rats. They reported a two fold increase in endurance exercise performance compared to matched controls. A similar approach using human subjects would be very useful, because it would not necessarily have any other effects in the CNS to confound the results.

1.8.2.5 The Proposed Mechanisms of Serotonin Mediated Fatigue

While there is ample evidence that a serotonin agonist will reduce exercise performance by inducing “central fatigue”, little is known about the mechanism of this fatigue. Previous investigators have assumed that central fatigue increases perceived exertion (Meeusen et al., 2001) and decreased neural drive (Bailey et al., 1992), which lead to a decrease in performance. These relatively safe assumptions are based upon the understanding of the structure and function of the network of serotonergic neurones in experimental animals (Jacobs and Azmitia, 1992). However, it is not know which of these two factors is responsible for the decline in exercise performance reported previously.

There are three reports of the effect of increased brain serotonin on perceived exertion in human subjects. Marvin et al. (1997) found that buspirone increased perceived exertion during the first half of a cycling test, but it was not different to the effect of a placebo at the point of fatigue (Figure 1.10). This finding is difficult to interpret because although the SSRI decreased performance perceived exertion was not increased at the point of fatigue. Therefore it makes it difficult to attribute the decrease in performance to an increase in perceived exertion. Struder et al. (1996) examined the effects of the administration of BCAAs and a saline treatment on treadmill exercise. They found no difference in rating of perceived exertion or exercise performance. A similar study by Meeusen et al. (2001) found no effect of a selective serotonin reuptake inhibitor (Fluoxetine) on perceived exertion at the end of an exercise time trial test, and also found no effect on performance.

[image removed]

Figure 1.10 Ratings of Perceived Exertion (RPE) during Exercise Following Treatment with the Serotonin Agonist Buspirone (Marvin et al, 1997). Filled points represent response to the agonist. RPE was significantly higher for the first 8 minutes of exercise.

A report by Cunliffe et al. (1998) examined the effect of an increase in brain serotonin on subjective and objective measures of fatigue. They stimulated an increase in brain serotonin by administering tryptophan (30 $mg.Kg^{-1}$) and found that this caused a significant increase in the ratio of free tryptophan to BCAAs. Subjects rated their own perceived fatigue on a visual scale and completed objective tests of fatigue each hour for four hours after tryptophan or placebo administration. The results of this investigation showed that perceived fatigue was significantly higher for tryptophan administration than the placebo response 4 hours post administration. The fact that these investigators did not measure endurance performance (cycling or running) during the period after tryptophan administration makes it difficult to determine what effect the increase in perceived fatigue would have on exercise performance. However they did measure reaction time, grip strength and wrist ergometric work each hour for 4 hours. The only significant effect

of tryptophan occurred at 4 hours post administration where there was a decrease in reaction time and a paradoxical increase in wrist ergometric work. Grip strength was unchanged by the administration of tryptophan at any time point. These contradictory findings do not help to explain why an increase in brain serotonin, simulated by the administration of drugs or tryptophan, decreases exercise performance. While there is some evidence of increased perceived exertion, there is no clear evidence of decreased neuromuscular function. Therefore this question of precisely how an increase in brain serotonin decreases exercise performance remains open.

1.9 Training Adaptations in the Serotonergic System

The CNS shares the characteristics of most biological systems in that it retains the capacity to adapt to repeated perturbations. The most common evidence of this is the typical increase in tolerance to the actions of drugs. Exercise training stimulates a range of changes to the structure and function of the heart and skeletal muscles in order to cope with the stress of intense or prolonged exercise. Reports of adaptations to exercise training in the CNS are rare, as its role as a determinant of exercise performance has not been recognised until recently. The central fatigue hypothesis however, has offered a rationale for the investigation of adaptations in the serotonergic system in response to exercise training. Potential adaptations fall into two categories, changes to the regulatory mechanisms of brain serotonin and alterations to the sensitivity of serotonin receptors, both pre and post synaptic. Adaptations effecting the concentration of brain serotonin may effect both the resting levels of serotonin and the response to exercise. Unfortunately there are no reports of changes to neurotransmitter release or direct measurements of the receptor population in human subjects. The data currently available is limited to

animal research and indirect measures of receptor function in humans. A report on elite athletes shows altered serotonin concentration in the blood, (Soares et al., 1994) and another more recent report shows an increase in the density of the serotonin transporter on platelet membranes of endurance trained athletes, but it is unclear what relationship these peripheral adaptations, if any, have to the CNS (Strachan and Maughan, 1998).

1.9.1 Serotonin Release During Exercise

The effect of exercise training on the concentration of brain serotonin at rest appears to depend upon the region of brain under examination. There are reports of an increase in the cerebellum, cerebrum and midbrain of rats (Brown et al., 1979) as well as the brain stem (Dey et al., 1992). Conversely a decrease in serotonin in response to training has been reported in the limbic forebrain (Hoffman et al., 1994) and hippocampus (Dey et al., 1992). Chaouloff et al. (1985) reported no training induced change in the post exercise concentration of whole brain serotonin. The response to training varies by brain region and this characteristic is also evident in reports that examine the response to acute exercise.

Training effects on the turnover of serotonin appear to be a reasonable indicator of the production of serotonin. The resting concentration of 5-HIAA is increased where the resting serotonin levels are also increased (Dey et al., 1992) and 5-HIAA is decreased where serotonin is decreased (Hoffman et al., 1994). The increase in resting serotonin is likely to be due to a chronic increase in the rate of entry of tryptophan into the brain because of changes to resting levels of plasma BCAAs or free fatty acids. Alternatively, presynaptic receptors may become desensitised with training and hence reduce their ability to inhibit any increase in the synaptic

concentration of serotonin. This response by auto receptors has been reported in central 5-HT1b receptors of rats after 5 weeks of exercise training (Seguin et al., 1998).

Training induced changes on the serotonergic response to an acute bout of exercise are critical to exercise performance. An attenuated increase in serotonin would be likely to delay the onset, or reduce the severity of, central fatigue. This attenuation could be achieved via an increase in the sensitivity of presynaptic auto receptors and/or an increase in the rate of degradation of serotonin into 5-HIAA.

There are contradictory reports of the effect of training on the serotonergic response to an acute bout of exercise. These reports include findings of an increase in serotonin (Dey et al., 1992; Brown et al., 1979), a decrease (Acworth et al., 1986, Hoffmann et al., 1994) and no change is brain serotonin concentration in rats (Chaouloff et al., 1985). Furthermore, the work by Dey et al. (1992) showed a variable serotonergic response depending upon the region of the brain. There are however, more consistent reports on the effect of training on the turnover of serotonin. There are three reports of an increase in the turnover of serotonin (Chaouloff et al., 1985; Ackworth et al., 1986; Dey et al., 1992) and only one report of a decrease (Hoffmann et al., 1994). Therefore, the weight of evidence appears to favour the claim that training leads to an increase in the turnover of serotonin in rats.

Training induced changes in the serotonin response to exercise are generally small and presently there are as many reports of an increase (Dey et al., 1992; Brown et al., 1979) as there are of a decrease (Ackworth et al., 1986; Hoffmann et al., 1994). Therefore the simple conclusion may be that there is probably no important change in the serotonin response to exercise after training. However the three reports that indicate an increase in 5-HIAA, suggest an increase in the turnover of serotonin.

One difficulty with these data is that, one would expect that training would lead to an increase in the rate of entry of tryptophan into the brain. Although this contention has not been tested in humans, it has been reported that trained rats have a larger increase in plasma free tryptophan during exercise than untrained rats (Chaouloff et al., 1985). This may be driven by an increase in the appearance of free fatty acids in the circulation. Therefore the increase in brain tryptophan should translate into an increase in the concentration of serotonin and a more pronounced central fatigue effect. However, the data does not support this. A possible explanation for this might be that the additional serotonin is metabolised into 5-HIAA, which prevents an abnormally large, increase in serotonin. If this is the case, then this adaptation would offer some protection from the effects of central fatigue.

1.9.2 Serotonin Receptor Sensitivity

The net effect of an increase in brain serotonin on central fatigue depends upon the magnitude of the increase of serotonin and the number and/or sensitivity of serotonin receptors. The previous section discussed the training induced changes in the serotonin/5-HIAA response to exercise and the data infer two important points i) the exercise induced increase in brain serotonin is augmented in trained rats and ii) the extra serotonin produced during exercise is degraded, preventing any abnormally large increase in serotonin. The possibility also exists of a decrease in receptor function to minimise the fatiguing effects of increased serotonin during exercise.

The possibility that receptor function plays an important role in the net effect of central fatigue is supported by experiments that have extended exercise performance by administering a serotonin antagonist to exercising rats (Bailey et al.,

1993a, 1993b). Because the blockade of receptor sites mimics down regulation of receptors, alterations in receptor function are likely to be beneficial to exercise performance. The interpretation of studies that examine training induced changes in receptor function is problematic because the selectivity of the drugs used is often poor (Dey, 1992) and the behavioural response to a serotonin receptor agonist does not necessarily reflect the "fatigue" response to a serotonin receptor agonist (Chaouloff, 1994).

Treadmill running for 3 and 5 weeks has been shown to decrease 5-HT1b (Seguin et al., 1998) and 5-HT1a (Dey, 1992) autoreceptor sensitivity in rats. This adaptation is counterintuitive, since it is likely to cause an increase in the release of serotonin into the synapse. The effect of training on post synaptic receptors is less clear with reports of no change in receptor sensitivity after 4 days of running training (Chaouloff, 1994) and a non significant decrease after 4 weeks of swimming in rats (Dey, 1992). Therefore, it is not possible to make any conclusive statements about training adaptations in experimental animals from these two reports.

Human studies provide preliminary evidence of down regulation of 5-HT1a (Jakeman et al., 1994) and 5-HT2c (Broocks et al., 1990) receptors in trained individuals. Both reports used the serum prolactin response to buspirone as an indirect measure of the sensitivity or number of serotonergic receptors, the so called "neuroendocrine challenge test". The blunted prolactin response was used as evidence for decreased serotonin receptor sensitivity in trained runners (Figure 1.11).

[image removed]

Figure 1.11 Comparison of the Prolactin Response to the Partial Serotonin Agonist, Buspirone, in Trained and Untrained Runners (Jakeman et al., 1994). The hollow circles represent endurance trained runners and the filled circles represent sedentary controls. * indicates a significant difference between trained and untrained subjects.

There were methodological limitations of these reports that make it difficult conclude that training causes a decrease in receptor sensitivity. Jakeman et al. (1994) did not use a placebo control to determine what proportion of the prolactin response was due to the effect of the drug. Stress, for example, is known to cause an increase in prolactin which may have influenced the results if it was higher in one group. Neither group of investigators actually trained their subjects, rather they compared trained runners with sedentary controls. The assumption made by these investigators was that prior training was responsible for the difference in receptor sensitivity. However, it is also possible that receptor sensitivity is inherently lower in some individuals, which may predispose them to athleticism. If this is the case, then the existing reports comparing trained and untrained cohorts do not provide conclusive evidence that training per se, causes a decrease in is serotonin receptor sensitivity.

Presently, the information regarding the existence and nature of training adaptations in the serotonin receptor of animals is incomplete. In addition, little is known about the time course of changes to serotonin receptors or whether the changes affect the onset of central fatigue during exercise and subsequent performance. The evidence regarding changes in receptor sensitivity in humans is circumstantial (ie. comparison of cohorts, not measuring changes pre-post training). A study is required to determine whether a period of training changes the sensitivity of post synaptic receptors in humans.

2.0 Decreased Serotonin Receptor Sensitivity in Endurance Trained Rats

2.1 Introduction

Changes in serotonergic function in response to acute bouts of exercise may provide information about the mechanism of central fatigue during exercise, however little is known about the chronic effect of exercise on this type of fatigue. Most organs involved in physical work (eg. muscles and heart) adapt to repeated bouts of exercise that offer significant physiological stress. The adaptations can be anatomical or biochemical and usually increase the capacity of the tissue to perform its function. The biochemical processes that limit endurance performance (eg. oxygen carrying capacity and blood buffering capacity) also adapt and offer improved resistance to fatigue. It is assumed that as training normally results in enhanced endurance performance and that endurance performance has been shown to be limited by serotonergic function in rats, the brain adapts to exercise training by delaying the increase in serotonin and/or reducing receptor sensitivity. The nature of the adaptations in the central serotonergic system is unclear, yet they may be as critical to endurance performance as those adaptations that occur in organs that are peripheral to the CNS.

In the few training studies of laboratory animals reported to date, decreased rates of production and increased degradation of serotonin within the CNS have been observed following endurance training (Ackworth et al.; 1986, Chaouloff et al., 1987). Although the changes reported were modest, exercise training was accompanied by improvements in endurance exercise performance. Therefore the changes in the rate of production and/or turnover of serotonin may not entirely account for the improvements in endurance performance, rather, serotonin receptor sensitivity may be partly responsible. However, it is not possible to quantify the contribution of changes in neurotransmitter turnover to improvements in endurance

exercise capacity. Reports of changes in serotonin receptor sensitivity after exercise training are rare. A report by Chaouloff (1994) found no change in receptor sensitivity after exercise training. Although, Chaouloff's study used only a short period of training (4 days) that may not have provided adequate stimulus for adaptation. Furthermore, behavioural characteristics rather than endurance performance were used to assess changes in receptor sensitivity.

The aims of the experiments described in this chapter were to use the serotonin agonist, m-chlorophenylpiperazine (m-CPP), to examine changes in serotonin receptor (5-HT1a) sensitivity during 6 weeks of exercise training in rats. In addition, experiments were designed to determine the time course of any exercise induced changes in the response to m-CPP and whether the response of individual animals to the agonist is predictive of their endurance exercise performance. It was hypothesised that endurance trained rats would show decreased sensitivity to m-CPP, thereby implicating the importance of down regulation of 5-HT1a receptors in the adaptive response to exercise training.

2.2 Materials and Methods

2.2.1 The Rodent Treadmill

The apparatus used in this study was a human treadmill modified for animal use. The running belt was divided into 6 separate corridors (150mm (w) x 650mm (L) x 150mm (H)) by suspending a box made of clear acrylic (Perspex^{TM}). This design allowed experimentation on 6 rats at the same time (Figure 2.1). The belt speed on the treadmill could be varied from 0.8-20 km/h in increments of 0.1 km/h. The accuracy of the speed was confirmed by manually measuring distance and time. Over a period of 10 minutes there was no more than a 2% error in speed.

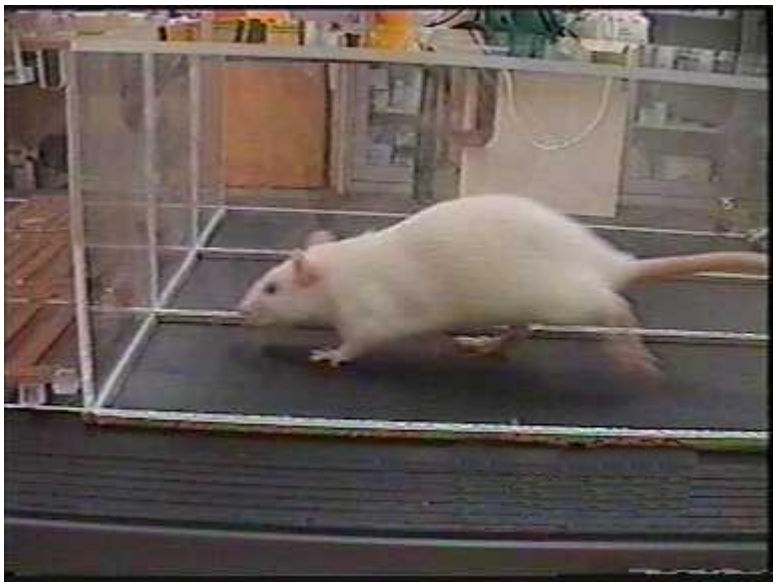


Figure 2.1 A Rat Running on the Rodent Treadmill

An electrified plate was positioned at the end of each corridor, level with the surface of the moving belt, to provide negative feedback to the rat (Figure 2.2). The rat was able to slide back onto the plate when it was fatigued and unwilling to run any further. The electric charge was delivered to a grid of copper tape, which was adhered to the plate in a matrix pattern. The spacing between the stripes on the grid was ~5 mm, which ensured that wherever the rat placed its feet, it would receive an

electric shock that caused some discomfort. This research was approved by the Griffith University Animal Ethics Committee.

Figure 2.2 Rats Resting on the Shock Plates After Exercise.
(The electric shock stimulus was turned off)

2.2.2 Training Regimen

The purpose of the present study was to produce a prompt increase in endurance capacity. The method of Chalouff et al. (1985) was modified to increase the number of days of exercise per week to 6 and to decrease the running speed to 0.8 km/h. The rationale for these modifications was to increase the total distance covered during training, without fatiguing the rats.

Thirty six Wistar rats were randomly allocated into three groups of twelve. It was discovered that some rats were unable or unwilling to complete the training regimen and were excluded from training and testing. Therefore the groups were as follows; a training group (T, n=11), a sham control group (SC, n=11) and a control group (C, n=10). All rats were housed in groups of 3 and experienced an inverse 12 hour day-

night cycle. They were given *ad libitum* access to food and water except when exercising.

The rats began training on the treadmill at 0.8 km/h and a 0% gradient for 15 min each day. The exercise duration was increased each day by 5 min until the exercise lasted 30 min. This gradual increase in exercise duration was required in the first week of training to allow the rats to become accustomed to treadmill running.

Rats in the T group trained 4 times per week for 6 weeks on the treadmill. Each training session comprised 30 min of running at a 0% gradient at 0.8 $Km.hr^{-1}$. Each week also included an endurance exercise test with and without the serotonin agonist, with a day of rest between.

Day	Test Group	Sham Control Group	Control Group
Monday	Train	Train	Train
Tuesday	Train	Train	
Wednesday	Train	Train	Train
Thursday	Train	Train	
Friday	Endurance test (no agonist)	Endurance test (no saline)	Endurance test (no agonist)
Saturday	Rest	Rest	Rest
Sunday	Endurance test (agonist)	Endurance test (saline)	Endurance test (agonist)

Table 2.1 The Weekly Training and Testing Regimen for Rats in Each Group.

The SC group was included to examine any effect the injection or test-retest interaction might have on the results. The group was treated the same way as the T group and completed the full training regimen, except that the serotonin agonist was replaced by saline.

The C group was included to examine any changes in receptor sensitivity that might occur independently of training (ie. due to maturation of the CNS during the experimental period). The C group performed one full week of training in the first week to allow the rats to become accustomed to treadmill running for exercise testing. On subsequent weeks they completed 2 days of training per week to maintain their relatively low trained state and otherwise underwent the same testing regimen as the T group.

Exercise time to fatigue, with and without m-CPP was assessed in all groups, at the end of each week. The T and C groups received m-CPP while the SC group received an equivalent injection of saline. Injection volumes were identical in each group.

2.2.3 Exercise Test Protocols

The requirements of the study were that the endurance exercise performance of the rats with and without the serotonin agonist was measured each week. An endurance exercise test without the serotonin agonist was performed on day 5 of each training week. Day 6 was a rest day and day 7 was a repeat of the endurance test, under the influence of the serotonin agonist (Table 2.1).

The endurance exercise test was a version of the protocol described by Bailey et al. (1992), modified to include a 2 minute warm up at a running speed of 0.8 $km.h^{-1}$. The speed was then increased over 30 s to 1.2 $km.h^{-1}$ and remained at this speed until the rats reached volitional fatigue. This point of fatigue was defined as the rat's preference for an electric shock on three successive occasions, over further exercise. This criterion was chosen to avoid the inclusion of test results from rats that rested on the plate before they were truly fatigued. Pilot testing revealed

instances of rats that would accept the electric shock after just 5 minutes of exercise, but when tapped by a finger, could run for a further 30 minutes. After the rat had returned to the shock plate for the third time, the current to that plate was turned off and the rat was allowed to rest. After a rest day (day 6) the rats repeated the same endurance test under the influence of the serotonin agonist (or saline in the SC group). This weekly regimen was continued for 6 weeks.

2.2.4 Serotonin Agonist Administration

A 5-HT1a agonist (m-chlorophenylpiperazine; m-CCP, Sigma Aldrich) was administered to evaluate exercise induced changes in the tolerance to this serotonin agonist. M-CCP is a potent 5-HT1a agonist (Leone et al., 1998) and mimics the effects of serotonin, having a dose dependent inhibitory effect on locomotion (Bailey et al., 1992; Gleason and Shannon, 1998). M-CCP has also previously been used in studies of 5-HT1a receptor sensitivity in rats (Bagdy et al., 1989; Leone et al., 1998). The dose (0.5 $mg.Kg^{-1}$) was corrected for body mass to minimise any confounding factors that might affect response to the serotonin agonist (eg. drug distribution and metabolism). M-CPP was diluted in normal saline (0.5 $mg.ml^{-1}$), warmed to 37^{o} C and injected intraperitoneally precisely 2 minutes prior to the commencement of the exercise test.

2.2.5 Statistical Analysis

Endurance exercise performance was measured as exercise time to fatigue (s). The suitability of group data for parametric testing was determined by the Kolmogorov-Smirnov test for normality. Comparison of group means was performed using the Jonkheere-Terpstra non-parametric ANOVA with post hoc comparisons by Wilcoxon Signed Rank test. Exercise time to fatigue is represented in the figures as group

mean ± SEM. Correlations were determined using the Spearman Rank Order correlation. Statistical significance was accepted where $p < 0.05$.

The study was designed to eliminate factors other than serotonin receptor sensitivity that might affect “drugged” endurance performance (eg. behavioural conditioning of the rat or improved “fitness” of the rat). In an attempt to allow for the existence of such confounding factors, the endurance data are also presented in a “normalised” fashion. In other words, the effect of m-CPP on endurance performance each week was normalised by expressing it as the percentage decrease from the undrugged performance in the same week (Figure 2.11).

2.3 Results

Several pilot studies were completed before the main study to describe several previously unpublished factors relevant to the methodology used in the present work. The factors examined were; the electrical stimulus required to make the rats run without causing any unnecessary cruelty, the dose and pharmacokinetics of m-CPP and the details of the rat training and testing protocols.

2.3.1 Pilot Studies

2.3.1.1 Electric Shock Stimulus

The present author was unable to find published data regarding the voltage and current required to provide negative feedback to exercising rats via their paws. Large voltages (10-100V) are required to overcome skin resistance, but small currents (~10 mA) provide adequate stimulus, (Casini, 1998). It was decided that the callous skin of the rat foot would provide more electrical resistance and hence the likely stimulus voltage would be ~100V. As the rat weighs only ~400 g, the stimulus current was reduced to ≤1mA. Experimentation on the investigator showed that an AC shock provided greater sensation than DC at any combination of voltage and current. An electrical circuit was produced that delivered a variable voltage (0-700 VAC) at a constant current of 0.5 mA.

An initial trial to test the efficacy of various voltages was made before the rats began regular training and testing. Most rats left the shock plate when the voltage increased to ~300V. There was variability between rats on this threshold voltage but 5 out of 6 rats would respond to 300V. This setting was used at the start of the pilot study to validate the training and testing regimen. It was increased to 500V after this

trial, when it was noticed that as the rats became accustomed to exercising on the treadmill their tolerance to the shock increased. The increased tolerance was probably due to thickened calluses on their feet and oxidation of the copper tape. The stimulus voltage was increased further to 600V after the first group of rats completed 6 weeks of exercise training. This voltage was required to cause the rat to leave the shock plate and continue running. The voltage was set at 600V for all further testing.

2.3.1.2 Drug Administration

It was important to determine the optimal dose to ensure that;

i. The serotonin agonist caused a measurable decrease in exercise time,
ii. This exercise time was short enough to exclude other fatigue factors (eg. substrate depletion) and
iii. Exercise time was not too short to make changes in receptor function difficult to detect.

After one week of training, six rats were injected with three different doses of m-CPP, to determine the optimal dose (0.5, 1.0 and 1.5 $mg.Kg^{-1}$). Three minutes after an intraperitoneal injection of m-CPP, the rats were required to begin the endurance exercise test described previously. The time to fatigue was recorded in seconds for each drug dose and is shown in Figure 2.3 below. The range of doses was selected based upon the work of Bailey et al. (1992) who described the dose response relationship of m-CPP for trained rats. The training regimen these authors used was different to that of the present study. A dose of 0.5 $mg.Kg^{-1}$ tended to reduce exercise time by the least amount (to ~4 minutes) which provided scope for both improvement and reduction in performance and was selected as the dose for the remainder of the study.

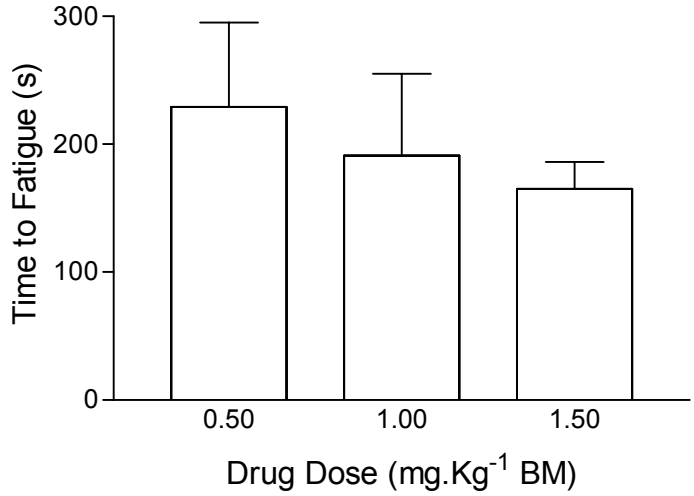


Figure 2.3 The Relationship Between the Dose of the Serotonin Agonist (m-CPP) and Endurance Performance. The results indicate a trend of decreasing performance with increasing dose (n = 6).

The delay between the administration of the serotonin agonist and the commencement of the exercise test was examined in a further pilot study. The serotonin agonist was administered (0.5 $mg.Kg^{-1}$) to six rats prior to an exercise test, on three occasions, employing a different delay each time. The exercise time to fatigue was recorded for each delay and is presented in Figure 2.4. There was no difference between each of the delay durations. A 2 min delay was arbitrarily selected as the standard because it reduced exercise time by the least amount, thereby allowing the greatest opportunity to measure further decreases in performance.

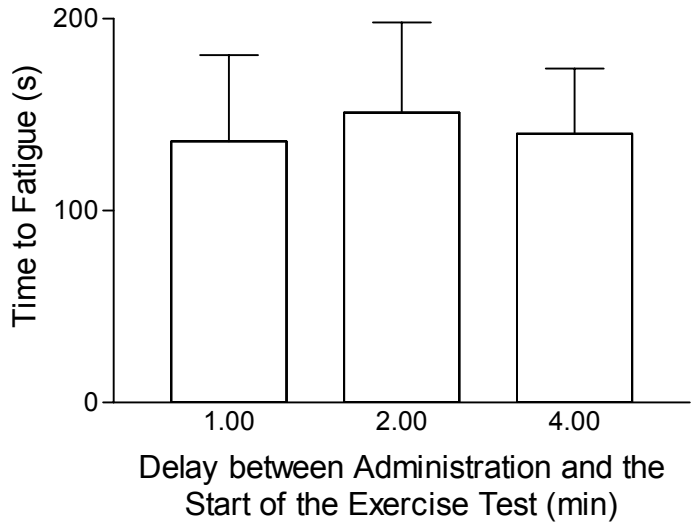


Figure 2.4 The Effect of the "Dose Delay" on Endurance performance. There was no real difference between a "dose delay" of 1, 2 or 4 min. and subsequent endurance performance (n = 6).

2.3.1.3 Training Regimen

The training regimen was validated by training a group of rats for 6 weeks to determine the rate of improvement in endurance exercise performance. After 6 weeks, there had been 4 consecutive weeks of significant improvement in endurance capacity, measured as time to fatigue. Hence this 6 week training regimen was adopted for the main study.

The order of treatments in any study is usually randomised, but it was not in the present study because it would have adversely affected the training regimen. In the pilot study, it was found that on the day after the long duration undrugged test, the rats were unable to complete the required training. It was assumed that this was due to residual fatigue, so the order of the endurance tests each week was always undrugged test first (day 5) followed by the drugged test (day 7). In the last two

weeks of training, a single day of rest was found to be inadequate to allow recovery from the endurance tests. The endurance tests lasted for more than 2 hours in some individual rats in these weeks and after only one day of rest, there was evidence of residual fatigue (ie. a decrement in performance from the previous week). Therefore in weeks 5 and 6, two rest days were allowed after the undrugged endurance test.

2.3.2 Main Study

In the Sham Control group, there was no significant difference between endurance performance with or without the saline injection in each week, with the exception of week 3 ($p = 0.041$) where saline treatment increased endurance performance (see Figure 2.5). This result indicates that the act of injecting the rat did not influence endurance performance. Thus, any decrease in endurance performance after the drug was injected was due to the action of the serotonin receptor agonist (m-CPP).

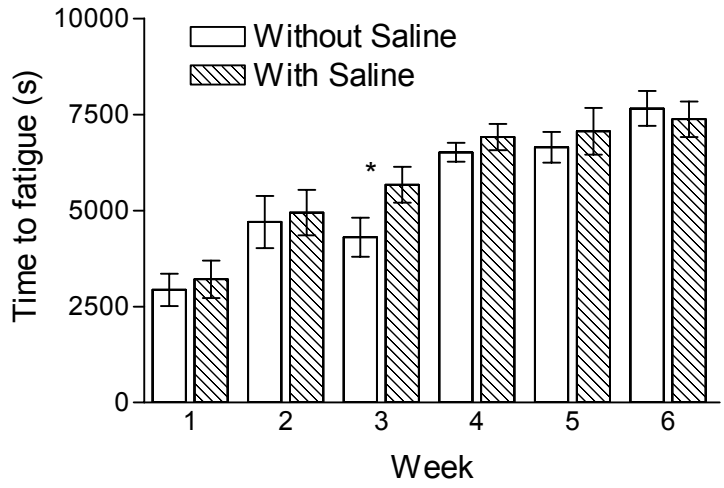


Figure 2.5 Endurance Performance in Trained Rats With and Without Saline. This figure indicates that a saline injection prior to an exercise test had no effect on endurance performance in all weeks except week 3. * indicates a significant difference in exercise time to fatigue within week ($p = 0.041$).

The endurance performance of the untrained control group remained relatively unchanged throughout the 6 weeks, with the exception of a decrease in performance in weeks 2 and 5 (Figure 2.6). Endurance performance with m-CPP treatment was consistent throughout training with a slight trend toward a decline in performance. There were small but statistically significant decreases in endurance performance in weeks 3 ($p = 0.017$) and 6 ($p = 0.005$) compared with week 1. Despite the differences in these two weeks, this result indicates that there was no effect of CNS maturation on the tolerance to the serotonin receptor agonist.

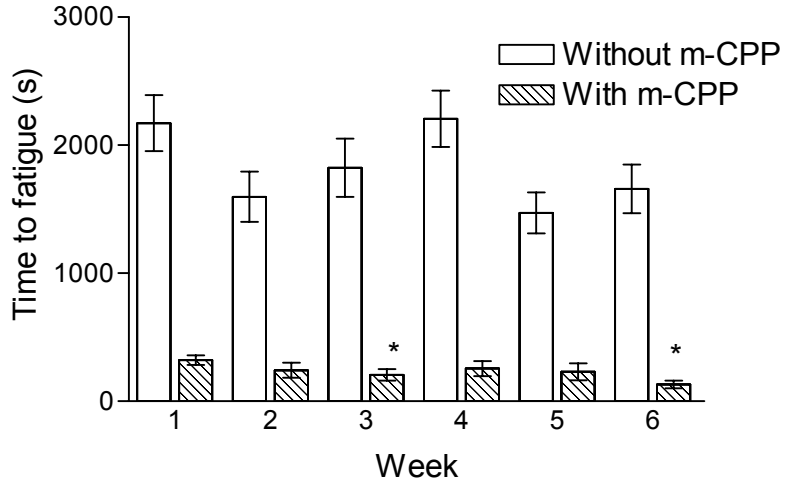


Figure 2.6 Endurance Performance in Untrained Rats With and Without m-CPP Treatment. Untrained rats completed an exercise test with and without the serotonin agonist (m-CPP) each week to examine any maturation effects on tolerance to the drug. * indicates a significant decrease compared to week 1 ($p < 0.05$).

A comparison of endurance performance after treatment with saline and the drug, in trained rats, demonstrates a significant difference in each week (Figure 2.7). Endurance performance after treatment with the drug is less than with saline, which clearly demonstrates the decrease in endurance performance following the administration of m-CPP.

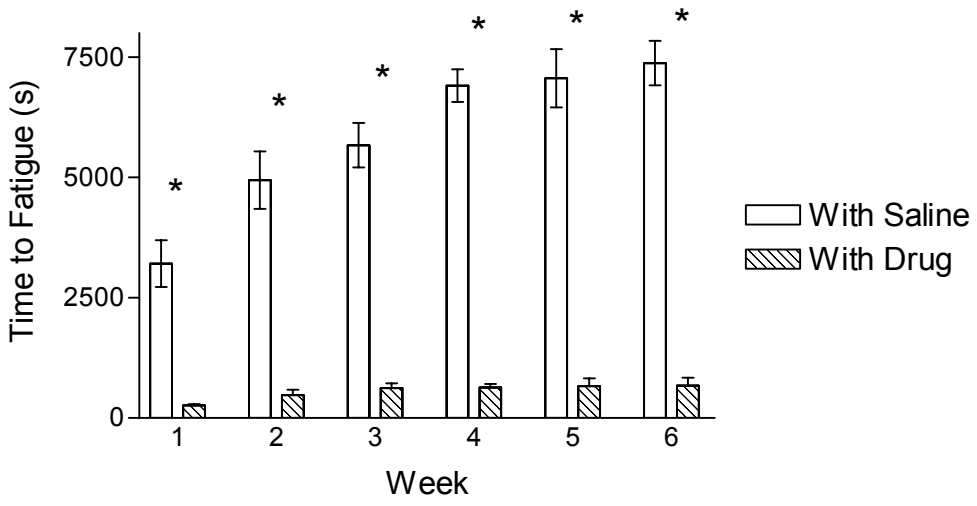


Figure 2.7 Endurance Performance in Trained Rats With Saline and With the Drug. The comparison of endurance performance with the serotonin agonist (m-CPP) and the vehicle (saline) indicates the effect of the drug on endurance performance. * indicates a significant difference ($p < 0.05$) between saline and drug treatment within each week.

Exercise training significantly increased endurance exercise performance in the Training group over the 6 week training period (4518 ± 730 s to 6640 ± 903 s, p = 0.01, Figure 2.8). Endurance performance in weeks 4, 5 and 6 was significantly higher compared to week 1. The significant increase in endurance performance confirmed that the training regimen provided adequate stimulus for physiological adaptations to occur.

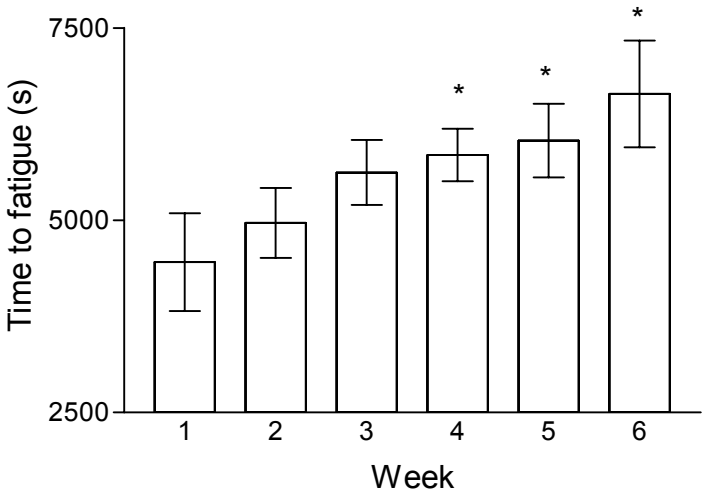


Figure 2.8 Endurance Performance in Trained Rats. Rats completed 4 training days and 2 testing days per week, for 6 weeks on a rodent treadmill. * indicates a significant difference ($p < 0.05$) compared to week 1.

Endurance performance under the influence of m-CPP also significantly increased in the Training group, after 6 weeks of training (260 ± 31 s to 679 ± 159 s, $p < 0.05$, Figure 2.9). This increase was significant ($p = 0.004$) at week 3 and then plateaued through to week 6. The significant increase in drugged endurance performance indicated that the rats were becoming tolerant to the fatiguing effect of the serotonin receptor agonist.

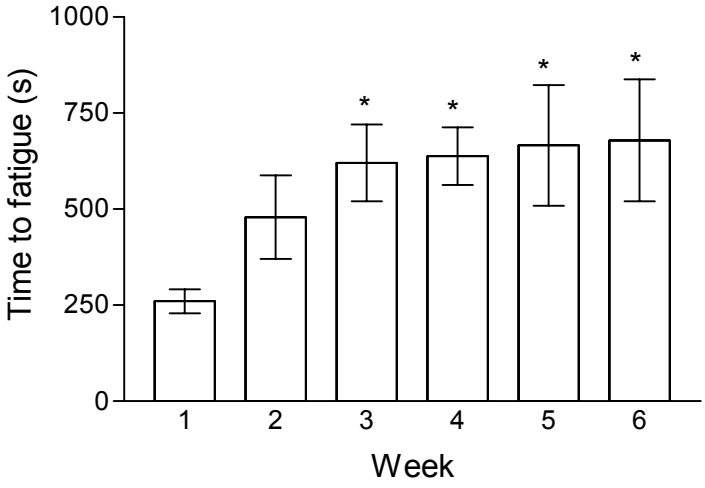


Figure 2.9 Endurance Performance in Trained Rats Treated with m-CPP. Rats were injected with 0.5 $mg.Kg^{-1}$ m-CPP prior to the exercise test in order to determine tolerance to the drug. * indicates a significant difference to week 1 ($p < 0.05$).

In order to clearly illustrate the effect of training, a comparison was made between the drug tolerance of trained and untrained rats (Figure 2.10). Tolerance to the drug is significantly higher in the trained group from week 3 to week 6, compared to the untrained group of rats. As the dose of the drug was kept constant throughout the training period, it is suggested that the increased tolerance occurred because of a decrease in serotonin receptor sensitivity.

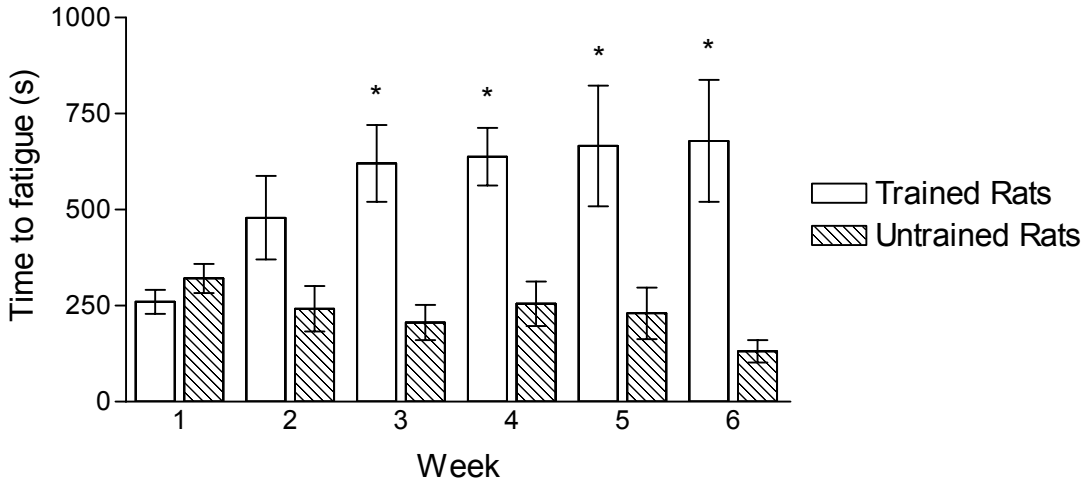


Figure 2.10 Comparison of Endurance Performance in Trained and Untrained Rats Treated with m-CPP (0.5 mg.Kg^{-1}). This comparison indicates the effect of training on tolerance to the drug. * indicates a significant difference between trained and untrained rats in the same week ($p < 0.05$).

To examine the effect of m-CPP on endurance performance, drugged performance was expressed as the percent reduction in undrugged performance in the same week. Normalising the drugged performances to the undrugged performance for each week, was intended to correct for any possible improvement in drugged performance that was due to any adaptations other than those of the serotonergic receptors. In the trained rats, the effect of m-CPP in week 3 is significantly less than in week 1 and this decrease is maintained until week 6 (Figure 2.11). On the other hand in untrained rats, the effect of m-CPP on endurance performance does not decrease, rather there is a slight trend of increasing effect.

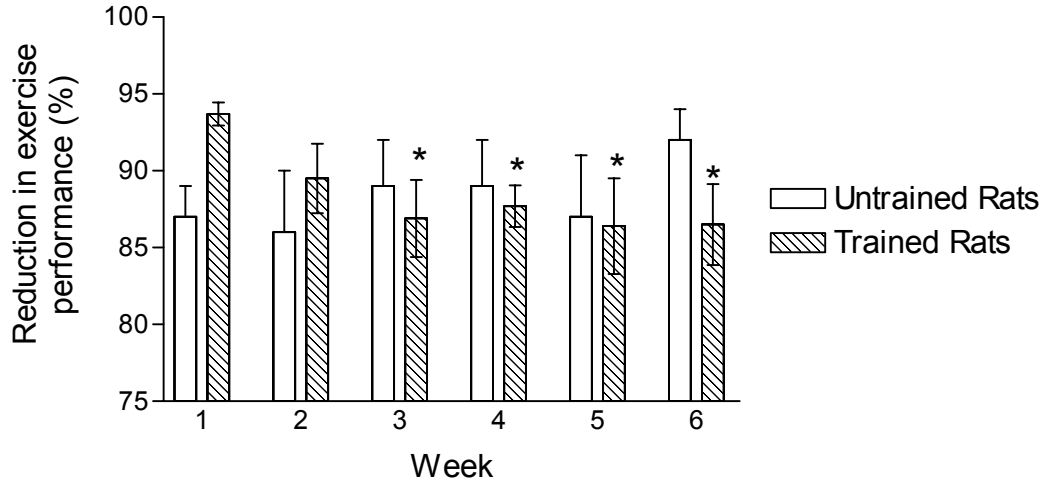


Figure 2.11 Effect of m-CPP on Endurance Performance in Trained and Untrained Rats, Normalised to Untreated Performance. Drugged endurance performance, expressed as the % decrease from the undrugged endurance performance in the same week, indicates improved drug tolerance in the training group and no change in the untrained group. * indicates a significant difference to week 1 ($p < 0.05$).

The ability of serotonin receptor sensitivity to predict undrugged endurance performance was determined by correlations of undrugged and drugged endurance performances in the T group for each training week. There were significant but weak correlations in weeks 1,2,3 and 6 (Table 2.2).

Training Week	1	2	3	4	5	6
Spearman's correlation coefficient	0.592	0.682	0.673	0.364	0.109	0.612
Significance (p=)	**0.027**	**0.010**	**0.012**	0.136	0.375	**0.030**

Table 2.2 Correlations Between Undrugged and Drugged Endurance Performance. Correlations are made for the T group only (trained and drugged). There was a significant correlation between undrugged and drugged endurance performance in weeks 1,2,3 and 6.

An example of the relationship between drugged and undrugged endurance performance is illustrated in Figure 2.12. Although the correlation was significant ($p = 0.02$), the strength of the correlation was weak ($r = 0.59$), indicating that tolerance to the serotonin agonist drug has some predictive ability of endurance performance.

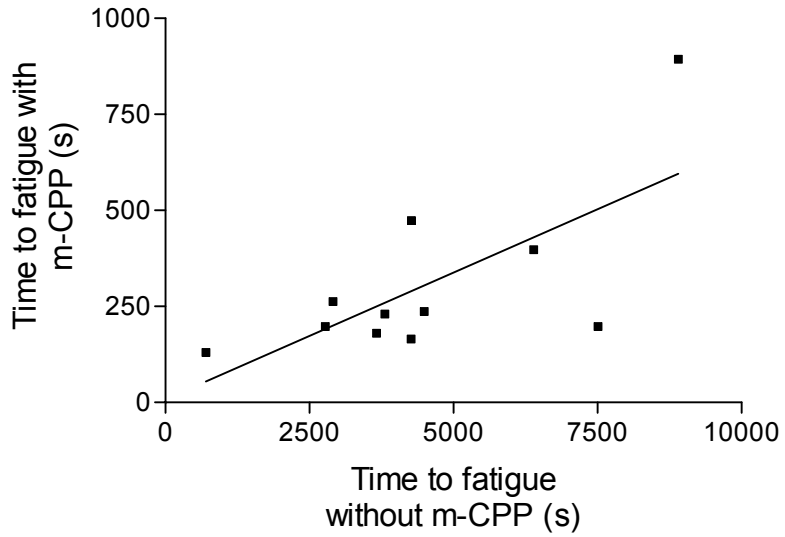


Figure 2.12 The Relationship Between Drug Tolerance and Endurance Performance in Week 1. Endurance performance with and without the drug are plotted each other to determine whether tolerance to the drug is predictive of endurance performance. There is a weak but significant correlation between drug tolerance and endurance performance ($r = 0.592$, $p = 0.027$).

2.4 Discussion

2.4.1 Design Considerations

The treadmill was chosen to train and assess endurance capacity in rats because of its versatility. Alternative exercise testing equipment includes a running wheel or a round pool of water for swimming. The running wheel offers the advantage of not needing an external stimulus to make the rat run. The rat runs on the outside of the wheel as a motor turns the wheel, and the rat will naturally avoid falling off. However it was felt that if the rat did fall off before it was truly fatigued, it would be difficult to immediately put it back on the moving wheel and make it run again. Furthermore, it is only possible to control running speed but not grade on a running wheel. A swimming pool offers an inherent stimulus for sustained exercise, however it is also not possible to vary the intensity of exercise. Both apparatus were thought to be unsuitable for the purposes of the present study.

The wistar rat was chosen for this research because of its wide use in comparable studies (review by Chaouloff, 1989). The rats started training at 9 weeks of age because the CNS of the rat has completed maturation by this age (Brown et al., 1979). This was important to avoid confounding exercise induced receptor changes with those attributable to maturation. The present results indicate that there is no change in receptor sensitivity attributable to maturation after 9 weeks of age in rats.

The choice of which serotonin receptor subtype to examine was a critical issue. There are several CNS serotonin receptor types, and a number of these have been implicated in the onset of fatigue during exercise (Ahlenius et al., 1997; Gleason and Shannon, 1998; Reith, 1990). However, the evidence from research that employs selective serotonin antagonists to isolate receptor subtype activity supports

the 5-HT1A receptor as having an important role in serotonin mediated fatigue, (Sills et al., 1985; Wikstrom et al., 1995; Beato et al., 1998). Additionally, the only previous study to report training induced changes in serotonin receptors examined 5-HT1a receptors. Therefore, a known 5-HT1a agonist (m-CPP) was used in the present work.

The possibility that peripheral adaptations to training may have affected endurance performance after treatment with m-CPP was considered. This confounding factor was minimised by normalising endurance performance with m-CPP treatment to endurance performance without m-CPP treatment in the same week. Additionally a drug dose was selected that limited endurance performance to such an extent that peripheral limitations to endurance performance (eg. substrate depletion) are unlikely to be involved in determining drugged endurance performance. In related studies, no contribution of peripheral factors to drugged endurance performance was found (Bailey et al., 1993b; Farris et al., 1998). The possibility that peripheral drug distribution might affect the efficacy of m-CPP, was also considered. Dose was corrected for changes in body mass in the present study and a previous study has reported no change in peripheral m-CPP metabolism after repeated administration (Ulrichsen et al., 1992).

2.4.2 Discussion of Results

The training regimen for the Training and Sham Control groups was sufficient to cause a significant increase in endurance performance. Treatment with m-CPP caused fatigue and a significant reduction in endurance exercise performance. The amount of fatigue produced, measured as a reduction in endurance performance, was progressively reduced during the 6 weeks of training. The likely reason for the

decreased effect of m-CPP is a decrease in receptor sensitivity. The decrease in sensitivity is probably in the hypothalamic 5-HT1a receptors, because of the selectivity of the agonist and the involvement of the hypothalamus in locomotion. The decrease in receptor sensitivity is probably due to the repeated increases in brain serotonin, induced by the exercise training regimen. The alternate explanation for this is that the change in serotonin receptor sensitivity was caused by the weekly administration of the serotonin receptor agonist. However, analysis of data from the sedentary control group reveals that weekly administration of the agonist, without any training, does not have any effect on receptor sensitivity. Therefore, the training alone is responsible for the decrease in receptor sensitivity.

The largest component of the total decrease in receptor sensitivity occurred in the first 3 weeks. From weeks 3 to 6 there was no further change in endurance performance under the influence of m-CPP. Thus when drugged endurance performance was normalised to undrugged performance in the same week, the pattern of change remained the same. This plateau in endurance performance may be due to the dynamics of 5-HT receptor regulation or to the design of the study. The dose of m-CPP varied in relation to the body mass of each animal, both of which increased throughout the training period. This was done to keep the effective dose of m-CPP constant, by allowing for changes in the pharmacokinetics of m-CPP, which are affected by body mass. Furthermore, it was assumed that any increase in body mass would have a consistent effect on the pharmacokinetics of m-CPP. However, the type of tissue responsible for the weight gain may not have been consistent throughout the training period (ie. initial skeletal muscle hypertrophy followed by increased adiposity). Therefore the dose of m-CPP would have been effectively increased, relative to the number or sensitivity of receptors, leading to the plateau in endurance performance. However, as there is little data on the pharmacokinetics of m-CPP, it is not possible to confirm this explanation.

The sham control group results indicate that the experimental protocol itself (ie. injection of saline) did not affect repeat test endurance exercise performance. There was no evidence of any effect of either the injection or residual fatigue from a previous test, on endurance performances. This confirms that any changes in endurance performance during drug treatment were due to the effect of m-CPP.

The primary role of the control group was to identify any decrease in receptor sensitivity over time and the results indicate that maturation was not responsible for a decrease in serotonin receptor sensitivity in 9-15 week old rats. The maintenance training completed by the control group did not increase drugged endurance performance, rather there was a decrease in performance in weeks 3 and 6. This may indicate an increase in receptor sensitivity associated with maturation but as the changes were not consistent across all weeks this is unlikely.

The relationship between drug tolerance and endurance exercise performance was determined to see if receptor sensitivity was predictive of endurance performance. The statistically significant correlations between undrugged and drugged performances in the training group suggest that tolerance to m-CPP may be predictive of endurance performance in weeks 1,2,3 and 6, although the correlations are weak (eg. week 1, $r = 0.592$). A previous report has shown that endurance trained athletes have lower receptor sensitivity when compared to sedentary controls (Jakeman et al., 1994). However the lack of consistency in the present results (in weeks 4 and 5) are confounding and weaken any conclusions that can be made from this study.

There are previous reports of down regulation of 5-HT1a receptors after chronic administration of the 5-HT1a receptor agonists, RU 24969, m-CPP and 5-MeODMT

(Oberlander et al., 1987; Ulrichsen et al., 1992; Sills et al., 1985) and of cortisol (Bagdy et al., 1989) in experimental animals. In human subjects, receptor down regulation after exercise has been reported for 5-HT2c receptors (Broocks et al., 1999), 5-HT1b receptors (Seguin et al., 1998) and in 5-HT1a receptors (Dey, 1994; Jakeman et al., 1994). On the other hand, Chaouloff (1994) found no change in 5-HT1a receptor sensitivity in rats after 4 days of exercise training. The results of the present investigation suggest that an adequate training stimulus is required for any measurable changes in receptor sensitivity to occur. At least 6-12 training sessions lasting 30 minutes or more are required. Furthermore, the method of assessment of receptor sensitivity is important. Chaouloff (1994) used behavioural measures of receptor function that may be insensitive to small changes in receptor sensitivity. Behavioural parameters (eg. frequency of flat body posture and fore paw treading), may not reflect changes in receptor sensitivity in the same way as changes in endurance exercise performance. In addition, any comparison between studies that employ different indices of receptor sensitivity is problematic.

The disparity in undrugged endurance performance in week 1 between the training and control groups suggests that the groups were not well matched (4518 ± 730 s vs. 2206 ± 330 s). The disparity is probably due to biological variability in the response of the rats to the exercise training and not an experimental error. Nevertheless, the main conclusions of the present study are based upon a comparison of drugged performances for these two groups, which were not different in week 1.

The analgesic effect of m-CPP may have influenced endurance performance. A reduction in the painful sensation provided by the shock plate may reduce the discomfort associated with strenuous exercise and increased exercise time to

fatigue. The precise effect of m-CPP induced analgesia on endurance performance in rats remains unknown and may be a confounding factor in the present work.

An increase in drugged endurance performance after 6 weeks of training in the Training group implies down regulation of central serotonin receptors. It is suggested that this adaptive change occurs as a result of repeated increases in brain 5-HT induced by exercise. A decrease in serotonin receptor sensitivity is likely to provide enhanced tolerance to the fatiguing effects of increased brain serotonin during exercise. This prediction is supported by the significant albeit weak, correlation between m-CPP tolerance and endurance performance. Since exercise is a stimulus for an increase in brain serotonin, the magnitude of alterations to receptor sensitivity after a period of training is likely to be dependent upon the duration, intensity and mode of exercise.

In conclusion, exercise training was shown to decrease sensitivity to the serotonin receptor agonist m-CPP, which is probably due to a decrease in serotonin receptor sensitivity. Furthermore, the time course of change in the sensitivity of serotonin receptors appears to be logarithmic in nature. The improvements in endurance performance with m-CPP plateaued after week 3 while untreated endurance performance continued to rise. This result suggests that the largest alterations to serotonin receptor sensitivity in rats occur in the early phase of training. The extent of receptor down regulation may be an important determinant of endurance capacity in an individual. This contention is supported by receptor blockade studies that have extended endurance performance in rats (Bailey et al., 1993b). Desensitisation of 5-HT receptors in some subjects may delay central fatigue and offer enhancements to endurance performance, however it is unclear how important this effect is in humans.

3.0 Effect of Exercise Training on Serotonin Receptor Sensitivity in Humans

3.1 Introduction

The contribution of serotonergic function to central fatigue is likely to be determined by both neurotransmitter concentration and receptor sensitivity. The effect of an acute bout of exercise on serotonergic function has been described in experimental animals (reviews by Chaouloff, 1997, Davis and Bailey, 1997). However, less is known about the chronic effects of regular exercise on serotonergic function. The published studies in this area have focused on changes in neurotransmitter turnover. There are reports of increased resting concentrations of brain serotonin (Brown et al., 1979), decreased post exercise concentrations of brain serotonin (Ackworth et al., 1986; Hoffmann et al., 1994) and increased degradation of brain serotonin (Dey et al., 1991) in endurance trained rats.

Reports on the effects of exercise training on receptor sensitivity are rarer still. The few studies available have shown that receptor sensitivity in exercise trained animals (Seguin et al., 1998; Dey 1994) and humans (Jakeman et al., 1994; Broocks et al., 1999) is lower than sedentary controls. It is hypothesised that the repeated increases in brain serotonin associated with exercise training, leads to an adaptive down regulation or desensitisation of central serotonin receptors. This decrease in receptor sensitivity may offer protection from the fatiguing effects of an increase in brain serotonin. Jakeman et al. (1994) showed that the neuroendocrine response to a 5-HT agonist (buspirone hydrochloride) in highly trained athletes was lower than sedentary controls, implying decreased central serotonin receptor sensitivity. A similar study was performed by Broocks et al. (1999) who also reported a decreased neuroendocrine response to a 5-HT2c agonist (ipsapirone),

but not to a 5-HT1a agonist (m-chlorophenylpiperazine), in endurance trained athletes. As the subjects in these reports were not trained during the investigation, it was not possible to determine whether decreased receptor sensitivity occurred as a result of exercise training or whether individuals were more likely to exercise due to inherent decreased receptor sensitivity and hence, to be considered more “athletic”. The data presented in the previous chapter, examined the effects of exercise training in rats and provided evidence of down regulation of central serotonin receptors. However, there are no similar reports of this occurring in human subjects in response to exercise training.

As central fatigue has been shown to be a limiting factor to endurance exercise performance, the effect of exercise training on central serotonergic function has implications for both athletic performance and general health. Increased serotonin receptor sensitivity is associated with depression (Dey, 1994) and chronic fatigue syndrome (Bakheit et al., 1992). If exercise training does cause a decrease in serotonin receptor sensitivity, it may offer an additional adjunct therapy for the treatment of depression.

The aim of the present investigation was to examine the effect of 9 weeks of exercise training on central serotonin receptor sensitivity in young male human subjects. Receptor sensitivity was measured indirectly by measuring the neuroendocrine response (Prolactin) to a partial 5-HT receptor agonist (buspirone hydrochloride). Receptor sensitivity was correlated with endurance capacity to examine any predictive capacity of receptor sensitivity for exercise performance. It was hypothesised that 9 weeks of exercise training would cause a decreased neuroendocrine response to a serotonin agonist, which would suggest a decrease in serotonin receptor sensitivity.

3.2 Materials and Methods

3.2.1 Subject Selection

Males (n=20) between 20-30 years of age were voluntarily recruited from the student population of Griffith University. All subjects were fully informed about all procedures and gave their written consent to undergo testing (Appendix B). This research was endorsed by the Griffith University Human Ethics Committee and conforms to the guidelines described in the Declaration of Helsinki (1989). A smaller age range could have been selected to increase homogeneity, however a larger range enhances external validity of the results. Additionally, rates of adverse reaction and interaction to the drug were increased in the presence of other medications and it was anticipated that the selection of a younger age group would minimise this effect. Subjects who had participated in regular exercise or sport within the last 6 months were excluded from the study to avoid any inherent effect of training on receptor sensitivity. There was the possibility that the female menstrual cycle might affect the metabolism of the drug and as such, females were excluded from participating in the study.

All subjects underwent testing to determine aerobic power, endurance performance and central serotonin receptor sensitivity. Receptor sensitivity was determined by measuring the prolactin response to a neuroendocrine challenge (Thompson et al., 1994). The group was divided into a training group (n=12) and a sedentary group (n=8). After nine weeks of training or inactivity, both groups repeated all tests to examine the effect of training.

Stage	Test Group	Control Group
1	Medical Screening	
2	Aerobic power and endurance tests	
3	Neuroendocrine challenge- drug or placebo	
4	Neuroendocrine challenge- placebo or drug	
5	9 weeks of training	9 weeks of no training
6	Neuroendocrine challenge- drug	
7	Aerobic power and endurance tests	

Table 3.1 The Testing and Training Regimen.

The subject selection criteria were designed in order to identify a group of people who were likely to; tolerate exercise training and testing well, exhibit a measurable improvement in aerobic and endurance capacity and offer the least risk of adverse reaction to a serotonin agonist.

3.2.2 Health Screening

Subjects that wished to participate in the research were assessed by a physician before the study began. There were two purposes for health screening each subject; to determine suitability for the exercise testing and training, and suitability for two <60 mg doses of the serotonin agonist (buspirone). Assessment criteria for suitability for exercise were at the discretion of the physician and conformed to the American College of Sports Medicine guidelines for exercise testing (Franklin, 2001). Tests were also included to identify subjects who presented a greater than normal risk of adverse reaction to buspirone and whose condition may affect the accuracy of the buspirone challenge test. Subjects underwent a liver function test, a full blood count, a resting electrocardiogram and a lung function test. All health factors that present a specific risk of adverse reaction to buspirone are reported in

the MIMS therapeutic guide (Carroll, 1999). These factors and other general health factors such as drug interactions were checked thoroughly with the medical history of each potential subject. Conditions that excluded subjects from taking buspirone and participating in this study are outlined in Table 3.2.

Condition
Abnormal liver function test results
Evidence of renal disease
Abnormal haematological parameters from a full blood count (HCT, Hb, WBC, MCV)
Abnormal resting ECG
The use of other drugs, which could cause interactions, such as MAOIs, SSRIs, neuroleptics and dopamine receptor blocking drugs.
A history of mental disorders such as depression
An illness that may affect endocrine function.
A history of alcoholism or any other substance abuse.
An endocrine disorder associated with hyperprolactinaemia.

Table 3.2 Conditions that Excluded Potential Subjects from Participating in the Study.

3.2.3 Drug Administration and Blood Sampling

Subjects reported for testing at 8:00AM after a 12 hr fast. An attempt was made to relax the subjects by accommodating them in a lounge with newspapers and magazines throughout the entire test period. A nurse inserted a 21 gauge cannula (Insyte^{TM}, Becton-Dickinson) into a forearm vein and a 3 ml blood sample drawn into a test tube (Vacutainer^{TM}, Becton-Dickenson) for baseline measurements. Each subject was then given an oral dose (tablet form) of either buspirone hydrochloride

(Bristol Myers Squibb), or an indistinguishable placebo with a glass of water. Each subject remained seated for 4 hours while a 3ml blood sample was taken every 30 minutes. After every alternate blood sample was drawn, a ~ 3ml backwash of normal saline was used to prevent blood from clotting in the cannula. For subsequent blood samples, the first 3ml of fluid was discarded before sampling whole blood. Blood was allowed to clot at room temperature (~20^{o}C) in the sample tube. It was then centrifuged for 8 minutes at 3000g and the serum was aspirated from the tube, placed into a labelled 1.5 ml test tube and stored at –70^{o}C for later analysis.

After the collection of 9 samples over 4 hours, each subject was given muesli bars and fruit juice. They were then driven to their home or place of work and instructed not to operate heavy machinery for the rest of the day.

3.2.4 Exercise Test Protocols

All subjects completed an aerobic power test and an endurance test, before and after 9 weeks of training. The aerobic power and endurance tests were performed on an electrically braked bicycle ergometer (Lode, Groningen, Finland). A familiarisation session on a day prior to the actual test was included to allow the subjects to become accustomed to the testing procedures.

The aerobic power test was performed with an electrocardiogram monitoring heart function during all exercise tests. The pneumotach and gas analyser in the metabolic cart used to measure gas exchange were calibrated before every test. The test protocol, hand signals to communicate exertion level and reasons to stop the test were explained to each subject before the test. Subjects were instructed not to exercise within 12 hours of the test and to fast for 3 hours prior to the test.

The aerobic power test began with a 25 or 50 W warm up for 3 minutes, followed by a 25 $W.min^{-1}$ ramp increase in workrate until volitional fatigue. The initial workrate was determined by the performance of each subject in the familiarisation session and their body mass. Heavy subjects (>80 Kg) who are likely to achieve higher peak power were given 50 W as a warmup workload. Subjects were instructed to select a cycling cadence between 70-80 $r.min^{-1}$ and to maintain it during the test.

The increase in workload was controlled automatically by a preset program in the bicycle ergometer. The subject was required to breathe through a small mouth piece with their nose sealed with a nose peg so that expired gases could be collected. Expired gases were analysed, breath-by-breath, for Ve ($L.min^{-1}$), VO_2 ($L.min^{-1}$) and VCO_2 ($L.min^{-1}$) by a metabolic cart (MedGraphics, CPX metabolic cart).

Each subject was given verbal encouragement during the test. An attempt was made to standardise this encouragement for all tests. The test ended when the subject was unable to maintain the required cadence (>60 $r.min^{-1}$). The test was followed immediately by a ~4 min warm down at ~50 W. The subject was then allowed to sit in a chair and recover. They were allowed to leave the laboratory when their heart rate had dropped below 100 $b.min^{-1}$ and they felt well.

Raw VO_2 data was divided into 20 s averages and the highest value was selected as VO_2 peak. The validity of this peak value was confirmed by examining the test results to ensure the subject had exercised “maximally”. The respiratory exchange ratio had to peak above 1.2 and evidence of a plateau in VO_2 was used to support the finding.

The endurance test required each subject to cycle for as long as they could at a workload that corresponded to 60% of their VO_2 peak. The cycling was performed on a Monarch 812e pendulum ergometer. The test began with a warmup at 25 or 50 W for 3 minutes @ 80 $r.min^{-1}$, and then increased gradually over 7 minutes to the target workload. The target workload was calculated using the formula;

Workload (W) = (Target VO_2 ($mL.min^{-1}$) – (3.5 x Body Mass (Kg)))/12

and then confirmed or adjusted by measuring VO_2 for 5 minutes after 10 minutes of exercise (Franklin, 2001). VO_2 was measured using the metabolic cart. Once the intensity had been set, the mouth piece and nose peg were removed for the remainder of the test. The test stopped when the subject could not longer maintain 80 $r.min^{-1}$ or where leg discomfort prevented them from continuing. Test time was used as an indicator of their endurance performance. The post training endurance test was performed at the same absolute workload as the pre-training test.

3.2.5 Exercise Training

Subjects were randomly assigned to either a training group (n = 12) or a control group (n = 8). The training group completed 3 x 30 minutes of cycling on an ergometer (Monarch, 812e) per week for 9 weeks. Each session was separated by at least one day and consisted of a 3 minute warm up at 40 W at 80 $r.min^{-1}$. The resistance on the ergometer was increased to a workload that corresponded to an oxygen consumption that was 70% of VO_2 peak. Subjects were instructed to maintain 80 $r.min^{-1}$ during all training sessions. This workload was verified or adjusted by measuring VO_2 for 10 minutes. The heart rate at 10 minutes was recorded (Beat, Polar, Finland) and used as a target throughout the 9 weeks of training. As the heart rate at the 10 minute mark decreased below the target heart

rate, the workload was increased to bring heart rate to within 5 $b.min^{-1}$ of the target heart rate. Subjects completed a 3 minute warm down at 40 W after training. Subjects in the control group were instructed not to perform any training or exercise during the same 9 week period.

After the 9 week period, both groups underwent the neuroendocrine challenge test and tests of aerobic power and endurance performance. All post training test conditions and procedures were preserved from the pre training tests.

3.2.6 Assay for Serum Prolactin

The concentration of serum prolactin was determined by radioimmunoassay (Immuchem Coated Tube, ICN Biochemicals, USA). Serum samples were analysed at the same time as a set of prolactin standards (0,2.5,5,10,25,50 and 100 $ng.dL^{-1}$) and a control sample. After the samples had thawed at room temperature and mixed gently, a 25 uL aliquot of each sample, the standards and the control sample were added to a tube coated with an antibody for prolactin and 200 uL of a reagent containing prolactin conjugated with $Iodine^{125}$. The tubes were vortexed and incubated for 2 hrs at $37^{o}C$, during which time the sample and the reagent competed for the antibody sites on the wall of the tube. The tubes were then emptied and washed twice with 1 ml of de-ionised water. The tubes were then placed in racks and assessed in a Gamma counter (1277 Automatic Gamma Counter, LKB Wallac, Finland). The emission of gamma radiation from each tube was counted for 1 minute (CPM). The standards allowed the construction of a standard curve using software (Excel, Microsoft), for the determination of Prolactin concentration. A control sample was included to determine the intra assay variability.

3.2.7 Statistical Analysis

The placebo was included to determine the Prolactin response to the stress associated with the test procedure (eg. venipuncture). To control for these factors, the Prolactin response to the placebo was subtracted from the Prolactin response to the serotonin receptor agonist. All figures, with the exception of the first one, have undergone this correction.

It was intended that the cross over design of the treatments (drug and placebo), be repeated after the training period. However, after the training period, the research subjects made it known that they were not willing to tolerate two more administrations of the drug. They were not aware of the presence of the placebo and had been told that they would receive the drug twice before and twice after training. Therefore the Prolactin response to the placebo was not assessed after the training period. The post training response to the serotonin receptor agonist was also corrected for by the placebo response obtained during pre-training testing.

The neuroendocrine responses were compared directly and as the area under the curve (AUC). AUC for each individual test was calculated as the sum of the product of prolactin concentration and sample period (always 30 minutes) at each of the seven samples taken after the administration of the drug.

All data were assessed for normality prior to analysis using the Kolmogorov-Smirnov test. Comparison of pre and post training data was made by a two way Repeated Measures ANOVA. Data are presented as mean $\pm$ SEM and statistical significance was accepted at $p < 0.05$. All figures that indicate the prolactin response to Buspirone have had the prolactin response to the placebo mathematically subtracted.

3.3 Results

One subject from the control group did not complete the study (n=7) while all subjects in the training group completed all tasks (n=12). All subjects remained unaware of the inclusion of the placebo in the neuroendocrine tests. The subjects generally tolerated the drug well. Some subjects experienced transient nausea but all subjects were asymptomatic within 6 hours of drug administration. The analytical coefficient of variation for the assay of serum Prolactin was 8.45%.

Table 3.3 Indicates changes in body mass, aerobic capacity, endurance capacity and the Prolactin response to the drug. The initial body mass of the two groups was not different ($p > 0.05$), nor was there any significant change in body mass as a result of training in either group. After training, aerobic power in the training group increased significantly (3.1 ± 0.2, to 3.6 ± 0.1 $L.min^{-1}$, $p < 0.005$), while it remained the same in the sedentary control group ($p = 0.36$). Endurance capacity also increased significantly in the training group (93 ± 8 to 168 ± 11 min, $p < 0.005$) but not in the control group ($p = 0.33$). These results indicate the training regimen achieved what it was designed to do, while the control group served its purpose well.

	Control Group		Training Group	
	Pre	Post	Pre training	Post training
Body Mass (Kg)	85 ± 3.1	87 ± 3.4	81.5 ± 3.9	80.7 ± 3.7
Aerobic power ($L.min^{-1}$)	3.3 ± 0.2	3.3 ± 0.2	3.1 ± 0.2	3.6 ± 0.1*
Endurance capacity (min)	98 ± 14	113 ± 21	93 ± 8	168 ± 11*
Area Under the Prolactin Response Curve ($ng.min.mL^{-1}$.)	6111 ± 1508	5677 ± 854	7647 ± 1509	7484 ± 1272

Table 3.3 Subject Characteristics, Exercise Capacity and Prolactin Response. There was no difference between groups for any measure in the “pre” condition. In the training group, aerobic capacity and endurance capacity increased significantly, while there were no increases in the control group. * indicates a significant difference to the “pre” condition ($p < 0.05$).

There was no significant increase in the neuroendocrine response to the administration of the placebo in either group (Figure 3.1), which indicates that the administration of the drug and the venipuncture procedures do not cause an increase in Prolactin. There was however, a small but significant decrease in Prolactin during 120-240 min post drug administration in the training group.

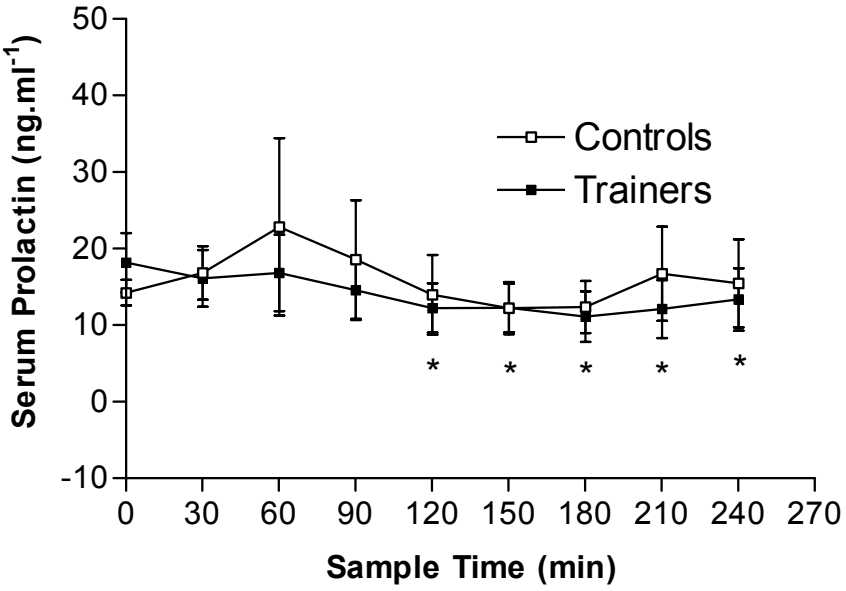


Figure 3.1 Prolactin Response to the Placebo in the Training and Control Group. The data indicate that venipuncture and the administration of a placebo does not cause a significant increase in Prolactin. * indicates a significant difference to 0 min in the Training group only ($p < 0.05$).

In the training group, buspirone administration caused a significant increase in serum Prolactin from 60-120 min compared to 0 min, both before and after the training period (Figure 3.2). However there was no difference between the pre and post training Prolactin responses, nor was there any change in the area under the prolactin response curve (7647 ± 1509 vs. 7484 ± 1272 ng.min.mL^{-1}, Table X.). These results suggest that the exercise training in the present study did not affect serotonin receptor sensitivity.

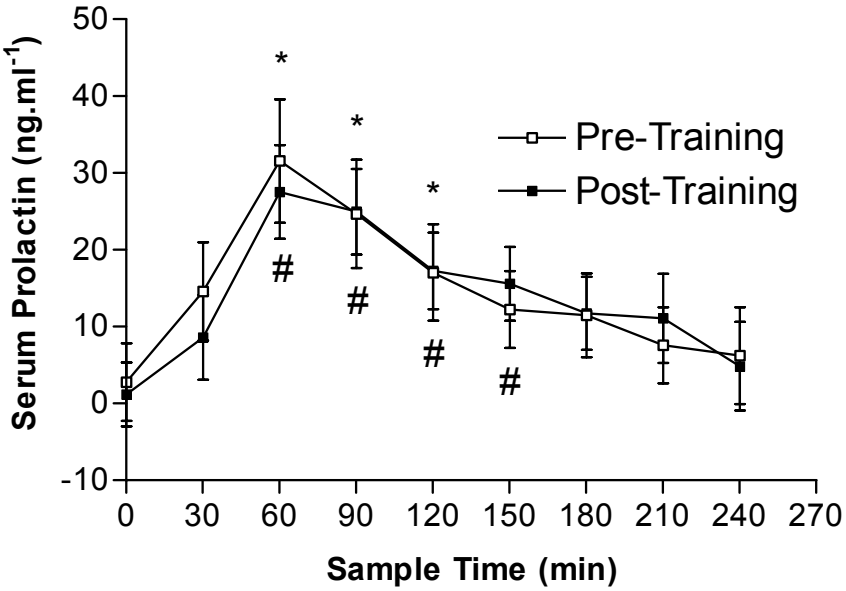


Figure 3.2 Prolactin Response to Buspirone in the Training Group. * indicates significant difference ($p < 0.05$) to the value at 0min for pre training data, # indicates significant difference ($p < 0.05$) to value at 0min for post training data. There is no significant difference between pre and post training data at any sample time, indicating that training does not affect receptor sensitivity.

In the control group buspirone administration also caused an increase in serum Prolactin, although the increase only reached statistical significance in the test conducted after the non-training period (Figure 3.3). There was no significant difference between the Prolactin response before or after training suggesting that there was no change in serotonin receptor sensitivity associated with any factors other than training.

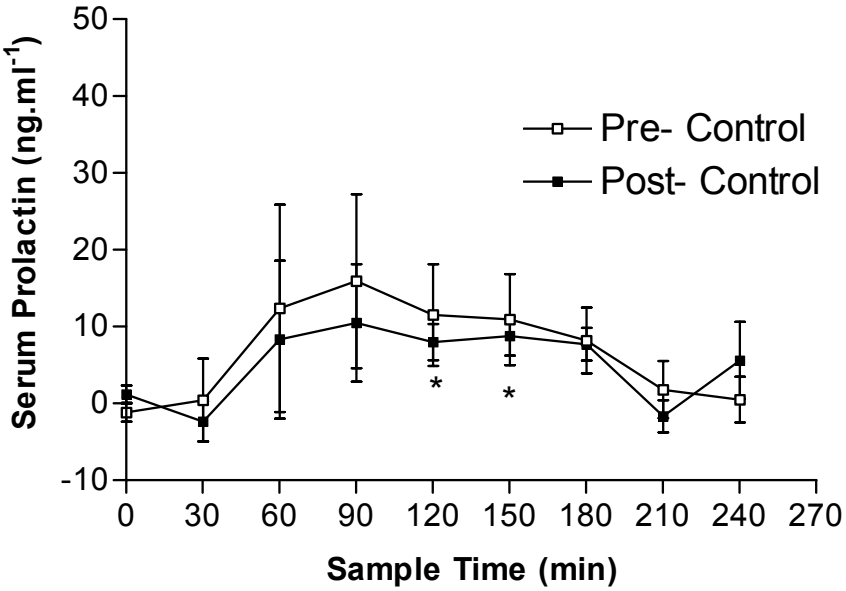


Figure 3.3 Prolactin Response to Buspirone in the Control Group. The control group was included to determine any background changes that may have occurred in receptor sensitivity. * indicates significant difference to 0 min.

Although there was no change in the prolactin response to buspirone after the training period for the group (Figure 3.2), there were 4 subjects (out of a total of 12) who demonstrated a large decrease in prolactin response after training (see Figure 3.4 for an example). These individuals may have responded uniquely to the training by down regulating their serotonin receptors.

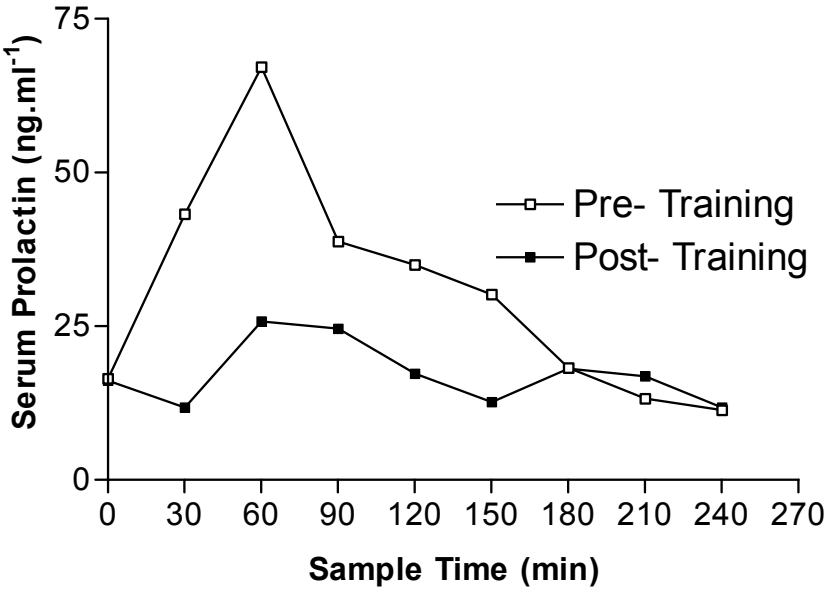


Figure 3.4 The Prolactin Response to Buspirone of an Individual. The decreased prolactin response to buspirone post-training indicates possible decreased serotonin receptor sensitivity.

Correlation between the Prolactin response (area under the curve and change in area under the curve) and exercise capacity (endurance capacity and change in endurance capacity) in the training group were calculated, in order to assess the ability of receptor sensitivity to predict endurance performance. There were no significant correlations between any combinations of variables.

3.4 Discussion

3.4.1 Design Considerations

It is not yet technically possible to directly assess receptor sensitivity in the intact human brain. In vivo techniques are available for use with experimental animals and simple radio labelling techniques can be used with biopsied brain tissue. Receptor sensitivity can be indirectly assessed by using a pharmaceutical (a serotonin agonist) that mimics the effects of endogenous serotonin. A large dose of a selective 5-HT agonist stimulates hypothalamic receptors that cause the anterior pituitary gland to release prolactin (Cowen et al., 1990). The magnitude of the rise and fall of prolactin over ~4 hours depends upon the dose of the agonist and the sensitivity of the receptors. While individual differences in rates of drug metabolism would also affect this, all subjects in the present work served as their own control. The dose was kept constant before and after the treatment, any change in the magnitude of the prolactin response was used as an indirect measure of a change in receptor sensitivity. The peak in prolactin or the area under the curve (measurements of serum prolactin concentration every 30 minutes for 4 hours post administration) can be used to assess serotonin receptor sensitivity. An attempt was made to reduce variance in the neuroendocrine response to the agonist by measuring prolactin in a fasted state at a consistent time of day.

This technique has been employed recently by other researchers who used the same pharmaceutical for the same or similar reasons (Jakeman et al., 1994; Meltzer et al., 1983, 1994). To the best knowledge of this author, there is no other safer technique for assessing the sensitivity of serotonin receptors in the human brain.

The present study required a selective serotonin receptor agonist that would produce a measurable endocrine response. Buspirone hydrochloride is a schedule 4 drug approved for therapeutic use in humans in Australia (manufactured by Bristol Myers Squibb). Over 1500 research papers published since 1983 have used buspirone hydrochloride for various purposes and more than 50 have measured the prolactin response to the drug. Buspirone has been used extensively for the purpose of determining the function of serotonin receptors (Bakheit et al., 1992; Sharpe et al., 1996). Additionally, the use of buspirone allows comparison with the only other study to have examined serotonin receptor sensitivity in athletes (Jakeman et al., 1994).

A limitation of the use of buspirone in the present work is that it is also a dopaminergic (D2) antagonist and this may be responsible for some of the prolactin release (Eison and Temple, 1986). Attempts have been made to determine the relative contribution of the serotonergic and dopaminergic systems to the release of prolactin with mixed results. At least two authors have concluded that agonism of serotonin receptors is entirely responsible for release of prolactin (Coccaro et al., 1990; Neuhauser et al., 1988), while another concluded that serotonergic function is not important (Maskall et al., 1995). The relative importance of serotonergic and dopamineric receptors in the release of prolactin is not clear. The problem with previous attempts to answer this question has centred on the difficulty of isolating the serotonergic effect of buspirone. Meltzer (1992) showed that the serotonin antagonist pindolol, blocked the prolactin release stimulated by buspirone. However Coccaro et al. (1992) demonstrated that pindolol has mixed antagonist and agonist actions on serotonergic receptors (1a) and that the work by Meltzer (1992) does not disprove the hypothesis that buspirone stimulates the secretion of prolactin via serotonergic receptors.

Alternative serotonin agonists to buspirone hydrochloride were considered. sumatriptan succinate (Imigran™) is used for the treatment of migraine headaches and is vaso active, causing changes in blood pressure and is associated with symptoms suggestive of myocardial ischaemia. Although the adverse reactions reported to occur with sumatriptan are described as mild and transient, they include chest pain, sensations of tingling, heat, heaviness, pressure or tightness in any part of the body, flushing dizziness and weakness. The published rate and severity of adverse reactions appear worse than that of buspirone and as such it was excluded on these grounds (Carroll, 1999). Most importantly, sumatriptan has not been established as a drug used for the assessment of serotonin receptor function. M-chlorophenylpiperazine (m-CPP) would have made a suitable agonist for the present work, but it is not presently available for use in human subjects in Australia.

The dose of buspirone administered to each subject was intended to fall within the recommended dosage limits and to produce a measurable increase in serum prolactin. Concern about the rates of adverse reactions was also a consideration. Buspirone can cause nausea, dizziness and hypotension. If these reactions were wide spread amongst the subjects they may decline further testing. The MIMS Therapeutic Guide (Carroll, 1999) suggests that the maximum recommended daily dose should not exceed 60 mg. However they also report, “In healthy normal human subjects the maximum tolerated dose of Buspar (buspirone) is 375 mg/day”. It was felt that the dose should also be corrected for body mass to allow for differences in the distribution of the drug and hence the neuroendocrine response. This method of weight corrected dosage also allows limited comparison between individuals of different body weight.

There are several research papers that have reported administering 60-100mg doses of buspirone for the purpose of measuring the prolactin response for the

same purpose as the present study (Jakeman et al., 1994; Bakheit et al., 1992; Seppala et al., 1987). Some of these studies used 60 mg per day over several days rather than a single dose, as used in the present study.

Other researchers have used smaller doses of buspirone (<50 mg) for reasons other than those related to the assessment of receptor sensitivity (Meltzer et al., 1983; Sharpe et al., 1996). At least one study that used buspirone for the same research purpose in a ~30 mg dose, found an increase in the prolactin release but was unable to find any difference in receptor sensitivity (Meltzer et al., 1994). Thus low doses of buspirone (~30 mg) were considered to be unsuitable for determining a valid neuroendocrine response and would be unsuitable for the present study.

As the heaviest subject in the present study weighed 100 Kg, a dose 0.6 $mg.kg^{-1}$ BM was selected as the dose. By doing so, the maximum dose of buspirone did not exceed 60mg in any subject either before or after the 9 week training period. Despite changes in weight after the training, the dose of the drug for each individual was not changed to allow comparison of prolactin response.

The possibility of introducing the subjects to a progressively stronger dose over several days reaching 60 mg was considered. This process would allow the subject to become tolerant of the drug and may be less likely to experience any side effects. However this would involve each subject using buspirone for 10 days (based on a recommended initial dose of 15 mg, increasing by 5 mg per day). It was felt that 10 days of buspirone administration posed a greater risk of adverse reaction, than a single dose did. Also, after this period of increased tolerance (ie. receptor sensitivity), it is likely that the technique will have lower sensitivity or lose validity all together.

Prolactin release is sensitive to many stressors; heat, exercise, emotional or physical stress and pain cause an increase in prolactin secretion (Thompson et al., 1994). A potential weakness of previous studies employing a neuroendocrine challenge to examine serotonin receptor sensitivity is the omission of a placebo control. It was anticipated that the stress or anxiety associated with taking the drug and venipuncture per se, had the potential to cause an increase in prolactin. A tablet, indistinguishable from the buspirone tablet, was manufactured from calcium for use as a placebo. Subjects underwent a neuroendocrine challenge twice before the training period commenced, once with the drug and once with the placebo, in a randomised order. The subjects were told that they would receive the drug on both occasions. Both the nurse administering the tablets and the subjects were blind to their content. The investigator was aware of what each subject received in the case of a serious adverse reaction.

3.4.2 Discussion of Results

The primary goal of the present investigation was to identify any change in the neuroendocrine response to a central serotonin agonist after 9 weeks of exercise training in healthy young males. A change would have been interpreted as a change in serotonin receptor sensitivity. The present hypothesis was based upon previous reports indicating that decreased central 5-HT receptor function enhances endurance exercise performance (Bailey et al., 1993b) and that exercise training reduces 5-HT receptor sensitivity in experimental animals (Seguin et al., 1998; Section 2.0). Furthermore, Jakeman et al. (1994) and Broocks et al. (1999) reported decreased sensitivity to serotonin agonists in elite athletes compared to sedentary controls. The suggestion from these last findings was that exercise training leads to a decrease in 5-HT receptor sensitivity. However, the present investigation found no

change in sensitivity to a serotonin receptor agonist after 9 weeks of exercise training and therefore, probably no change in central serotonin receptor sensitivity.

The assessment of human central serotonin receptor function is presently restricted to indirect methods and there are few serotonin agonists that are available for human experimentation. Buspirone was chosen for its safety and because it offered an opportunity to make comparisons with previous research that employed this drug. However, buspirone's lack of selectivity for 5-HT receptor subtypes makes it difficult to isolate the receptor/s responsible for the neuroendocrine response it stimulates. Buspirone has been shown to be a partial agonist of 5-HT and an antagonist of dopamine receptor subtypes and the prolactin response to buspirone may be mediated by more than one receptor (Maskall et al., 1995). A survey of the literature reveals a short history of sometimes contradictory data that implicates and excludes many 5-HT subtypes with regard to their involvement in prolactin secretion. Recent evidence implicates the 5-HT2a, 5-HT2c and 5-HT3 receptors while there is evidence both for (Porter et al., 1999) and against (Meltzer et al., 1992) the role of 5-HT1a receptor. The role of 5-HT2 receptors appear to have had the most consistent support in the literature and are the most likely, but not exclusively, to be involved in prolactin secretion (Nash and Meltzer, 1988; Di Renzo et al., 1989; Van de Kar et al., 1989, 2001). Therefore the neuroendocrine response in the present investigation probably represents the sensitivity of 5-HT2 receptors and that the exercise training does not alter 5-HT2 receptor sensitivity. However, changes in dopaminergic receptor sensitivity remain a complicating factor.

The present results can be contrasted to those of Jakeman et al. (1994) and Broocks et al. (1999) who found a decreased prolactin response to a serotonin agonist (ipsapirone) in athletes compared with sedentary controls. Although it is difficult to make a clear comparison as the design of the studies reported by these

authors is different to the present investigation. The failure of the present study to achieve the same result may be because the training stimulus in the present work was inadequate. Although, one quarter of the training group responded by demonstrating a decrease in sensitivity to buspirone while none showed an increase. A continuation of the training period beyond 9 weeks would have provided a greater stimulus and may have increased the number of "responders". There are two alternative explanations. Certain individuals may self-select for athleticism because they have inherently low receptor sensitivity, or they have an enhanced ability to lower their receptor sensitivity. Either characteristic may offer additional protection from the fatiguing effects of serotonin and increase the likelihood that an individual will become an athlete. While the present investigation does not substantiate this claim of inherently lower receptor sensitivity, it does lend support to possibility that some people may be considered "responders". Four of the twelve subjects who trained in the present study, demonstrated a substantial decrease in receptor sensitivity. While further work is required to support these contentions, they may explain the disparity between the present findings and those of Jakeman et al. (1994) and Broocks et al. (1999).

Previous animal training studies have reported significant decreases in receptor sensitivity after 5 (Seguin et al., 1998) and 6 weeks (Chapter 2.0) of exercise training. It appears from the present results that if humans are able to down regulate serotonin receptors in response to exercise training, the period of adaptation is significantly longer than 9 weeks. The duration of each training session may also be an important determinant of the magnitude of the adaptation, although the precise duration of exercise required to elicit an increase in brain serotonin in humans is not known.

Examination of central serotonergic function in rats has shown an attenuated exercise induced increase in 5-HT as a result of training (Hoffmann et al., 1994). Other authors have reported increased concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) (Ackworth et al., 1986). These results may suggest that training causes an increase in the rate of removal of synaptic 5-HT, however it is unclear whether the increase in 5-HIAA is due to increased exercise intensity and/or duration that is associated with training adaptations. Nevertheless, alterations in pre-synaptic receptors may also play a role in training adaptations in the central nervous system. An attenuated exercise induced increase in 5-HT may be explained by the up regulation of pre-synaptic 5-HT receptors (Seguin et al., 1998). This type of adaptation may decrease the concentration of synaptic 5-HT and possibly reduce the fatiguing effects of serotonin during prolonged exercise.

The placebo control was included to measure the neuroendocrine response to the stress and anxiety of drug administration and blood sampling. However, the resting Prolactin concentration was within the normal range (<20 $ng.mL^{-1}$) for placebo and drug administration. Furthermore, there was no significant increase in serum Prolactin after placebo administration for either group. There was actually a small decrease in Prolactin after 120 min in the training group, which probably reflects normal diurnal rhythm. The control group was recruited to examine any potential seasonal changes in serotonin receptor sensitivity. As there was no change in the neuroendocrine response to the agonist, any changes in receptor function that may have occurred were smaller than the sensitivity of this method.

In conclusion, the training protocol in the present study caused significant increases in aerobic power and endurance performance. The increase of 0.5 $L.min^{-1}$ in aerobic power in the training group is similar to that reported in a study with a comparable training regimen (Mitchell et al., 1996). Adaptations to aerobic conditioning may

include increased blood volume, cardiac output, muscle mitochondrial volume and muscle glycogen content. These adaptations, which are peripheral to the central nervous system, are the most likely explanations for the increased endurance performance seen in the present study. The lack of any change in serotonin receptor sensitivity may be due to a failure of the training protocol to provide adequate stimulus for an adaptation to occur.

4.0 Decreased Neuromuscular Function in Response to a Serotonin Receptor Agonist in Humans.

4.1 Introduction

The effects of an increase in brain serotonin on exercise performance have been described and there is some understanding of the role of serotonin in central fatigue. Attempts to artificially simulate the effects of exercise-induced fatigue in humans have been made by administering selective serotonin reuptake inhibitors (Wilson and Maughan, 1992; Meeusen et al., 2001), serotonin agonists (Marvin et al., 1997) and tryptophan (Cunliffe et al., 1998). With the exception of one report (Meeusen et al., 2001), an artificial increase in brain serotonin has been shown to cause fatigue during exercise. Similar methodology has also been employed in experimental animals where Bailey et al. (1992) demonstrated a near linear relationship between the dose of a serotonin receptor agonist (mCPP) and the reduction in exercise performance in rats, measured as running time on a treadmill (Figure 1.8). Further work by Bailey et al. (1993b) showed a dose-dependent decline in treadmill running performance, under the influence of another serotonin agonist (quipazine dimelate).

To date, there is less understanding of the specific effects of an increase in brain serotonin on neuromuscular function in humans. It has been assumed that the mechanism of fatigue that leads to a decrease in performance during locomotion type exercise, involves a reduction in the ability of the muscles to produce sufficient force (ie. decreased neuromuscular function). There are several reports on the effect of a single dose of serotonin agonist on psychomotor performance that provide conflicting results. For example, separate studies have shown that buspirone has no effect on reaction time and that it can slow reaction time (reviewed

by O'Hanlon, 1991). There are two reports examining the effect of an artificial increase in brain serotonin and muscle strength. Schaffer and Klausnitzer (1988) found that 5 mg of buspirone had no effect on hand grip force or electromyogram signal amplitude. Cunliffe et al., (1998) described the effect of tryptophan supplementation on grip strength and ergometric work performed by the wrist. They found a non-significant decrease in grip strength and a paradoxical increase in wrist ergometric work. These findings are contradictory and complicated by the fact that there are methodological differences between these studies (eg. different drugs and doses). The mechanism by which serotonin decreases exercise performance is further confused by the fact that the serotonin agonists used in previous studies may have caused fatigue during exercise via other effects such as a decrease in cardiac output (Bagdy et al., 1989) and an increase in perceived exertion (Marvin et al., 1997). Therefore, it has not been established that an increase in brain serotonin causes a decrease in exercise performance because of its detrimental effects on neuromuscular function.

The aim of the present investigation was to artificially increase brain serotonergic activity using a partial serotonin agonist (buspirone), and examine its effect on five measures of neuromuscular function, including; reaction time (RT), hand eye coordination (HEC), isometric neuromuscular control (INC), maximal voluntary isometric contractile force (MVIC-F) and isometric muscular endurance capacity (IMEC). It was hypothesised that the serotonin agonist would produce changes in all five measures of muscle function that are consistent with exercise induced fatigue.

4.2 Materials and Methods

4.2.1 Subject Selection

Ten healthy male subjects, 20-30 years of age, were recruited from the student population of Griffith University. Gender and age were restricted to achieve a relatively homogeneous group. Females were excluded due to the possible effect of the menstrual cycle on drug metabolism. The group age range was arbitrarily confined to 20-30 years of age. There was no restriction on training status or training history included in the selection criteria. All subjects were fully informed about all procedures and gave their written consent to undergo testing (Appendix E). This research was approved by the Griffith University Human Ethics Committee and conforms to the guidelines described in the Declaration of Helsinki (1989). Prior to the commencement of the study, a physician determined the suitability of each subject for administration of the serotonin receptor agonist (buspirone hydrochloride), via a medical history and an assessment of normal lung, cardiac and liver function. Subjects who presented any extra risk of adverse reaction to the drug, determined by the presence of exclusion criteria, were not entered into the study. The exclusion criteria are outlined in Chapter 3 in Table 3.2.

4.2.2 Test Protocols

A set of tests were designed to assess five measures of neuromuscular function that may be affected by central fatigue and also have the capacity to decrease exercise performance (Table 4.1). An increase in brain serotonin was simulated by the administration of a serotonin receptor agonist (buspirone hydrochloride). The

tests were run in a sequence and short rest periods were allowed between each test. The order and duration of all tests was kept constant throughout the study.

Commencement Time (h:m)	Test
0:00	3 x Reaction Time (total of 15 attempts)
0:05	3 x Hand Eye Coordination (total of 30 attempts)
0:10	3 x Maximal Voluntary Isometric Contraction (3 s)
0:20	3 x Isometric Neuromuscular Control (15 s)
0:25	1 x Isometric Muscle Endurance Capacity (30 s)

Table 4.1 The Battery of Neuromuscular Function Tests.

The effect of the drug on neuromuscular function was determined in a cross over design with placebo control. Each subject performed the set of five tests, a total of four times in the study. The five tests were performed both before and after, administration of the serotonin receptor agonist and the placebo. The order of the treatments was randomised throughout the group and at least two days separated each treatment (Table 4.2.)

Time (h:mm)	0:00-0:25	0:25-0:30	0:30-1:30	1:30-1:55
Session 1	Test Battery	Placebo	Rest	Test Battery
At least two days rest				
Session 2	Test Battery	Drug	Rest	Test Battery

Table 4.2 Test and Treatment Sequence.

Prior to the first testing session, subjects were familiarised with all procedures and given the opportunity to practice the tests. Subjects were instructed not to perform any strenuous exercise in the 24 hours preceding the test day and to fast for three

hours prior to testing. All subjects performed the following two computer tasks seated comfortably in a position that was kept consistent throughout all tests. The room was quiet during all testing to avoid distracting the subject.

4.2.2.1 Reaction Time (RT)

RT was determined using customised software (LabView, National Instruments). Subjects were required to press a button in response to the illumination of a large red light on a computer screen (Figure 4.1) that was illuminated at random intervals (similar to the method described by Cunliffe et al., 1998). RT was calculated as the average time (ms) taken to respond to the illuminated light over a total of 15 attempts. An increase in reaction time was considered to represent a decrease in performance.

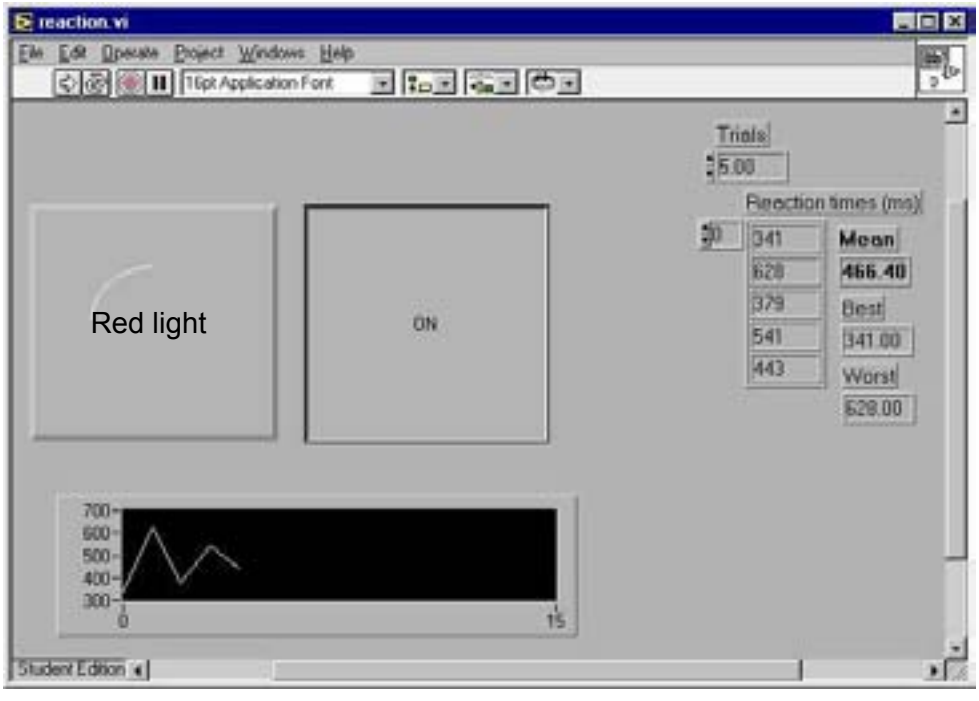


Figure 4.1 The Interface of the Reaction Time Testing Software.

4.2.2.2 Hand Eye Coordination (HEC)

HEC was also determined using customised software (LabView, National Instruments), which required the subject to use a computer mouse to move a pointer and click on a target as quickly as possible when it appeared in a random position within a defined area (15 x 15 cm) on a computer screen. The target was a red square button (1.5 x 1.5 cm) that had to be clicked with the computer mouse button when it appeared. After each click, the target square would immediately move to another random position. HEC was calculated as the average time (ms) taken to move the pointer and click on the target red square, calculated over 15 attempts. An increase in response time was considered to represent a decrease in hand eye coordination.

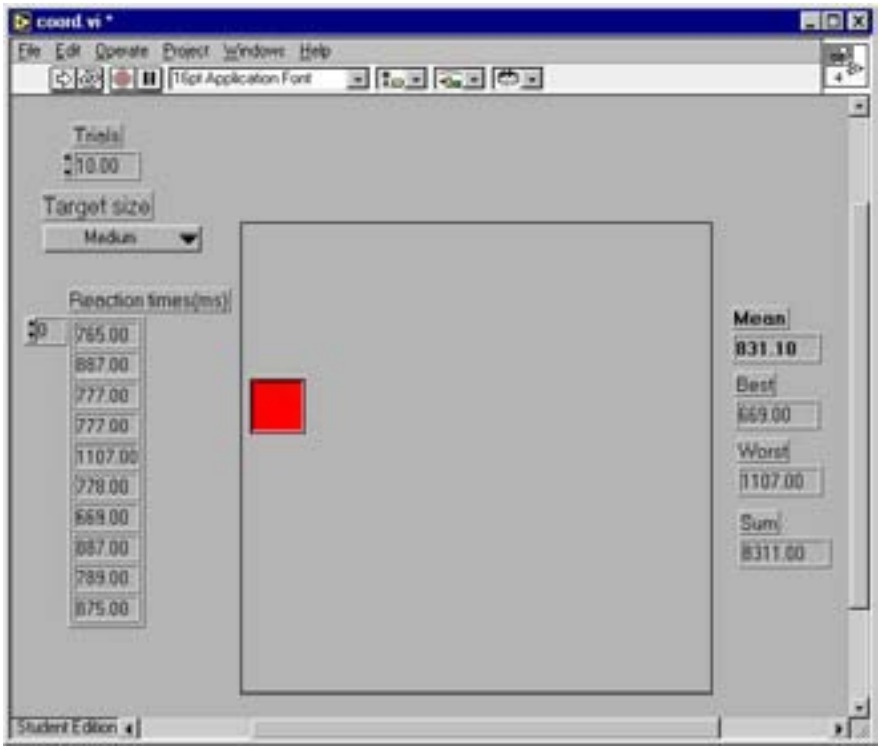


Figure 4.2 The Interface of the Hand Eye Coordination Testing Software.

4.2.2.3 Measurement and Data Acquisition Hardware

During the following three tests, subjects were seated in a purpose built machine (modified “Preacher curl” bench, see Figures 4.3 and 4.4) that isolated the movement of the forearm. Subjects sat with their dominant arm supported by a declining padded shelf, mounted in front of them. Their non-dominant hand was held behind their back and their feet placed comfortably behind them to avoid the use of their legs in any test. Subjects were required to flex their elbow while holding a handle that was tethered to a fixed point in front of them. The sitting position minimised the influence of all muscle groups other than the elbow flexors and was intended to keep the elbow joint angle at 90^{o}. This position was consistent for all subjects throughout the testing procedures. Subjects were instructed to use their elbow flexors only and tests were repeated if there was any evidence of a deviation from the standard technique.

Contractile force was measured by a 1 kN S-type force transducer (Applied Measurement, Australia) that was connected in series with the handle. The transducer signal was amplified with a gain of 1000 using MP100 BioPac hardware (Santa Barbara, USA) and recorded using Acknowledge data acquisition software (National Instruments, USA) sampling at 1000Hz. The force transducer was calibrated with known weights prior to all testing so that the software could convert the signal from the amplifier to a force (N).

Figure 4.3 Diagram of the Exercise Machine.

Figure 4.4 A Subject Using the Exercise Machine.

4.2.2.4 Maximal Voluntary Isometric Contraction Force (MVIC-F)

MVIC-F was measured whilst the subjects performed a three second maximal isometric bicep curl sitting in the custom made preach curl bench. Subjects were told that the accuracy of the test depended on their ability to flex their elbow maximally and they were given three attempts. MVIC-F was determined as the maximum force (N) achieved between 0.5 and 2.5 s, from three tests (Bigland-Ritchie et al., 1986a).

4.2.2.5 Isometric Neuromuscular Control (INC)

INC was defined as the ability to control a submaximal isometric contraction for a 15s duration at a target force corresponding to 30% of the subject's MVIC-F (Krogh-Lund and Jorgensen, 1993). A computer display, positioned so that the subject could maintain their position in the preacher curl bench, featured a large graph of the subject's current force production and the target force production. The subject was instructed to sustain a 15 s contraction as close to the target force as possible, using the graph to provide real time feedback on their performance. An initial attempt of ~10 s was permitted to allow the subject to become familiar with the target force and the technique. This test was performed a total of three times with a rest of 1-2 minutes between attempts.

Isometric neuromuscular control was assessed by calculating the statistical variance of force, with respect to the target force, for the last 10 s of the 15 s test. The first 5 s were deleted to remove the initial rise in force to reach the target force. The formula for variance (σ^2) of a population sample was modified by exchanging, sample mean for target force.

$$\sigma^2 = \frac{\Sigma(X - x)^2}{n}$$

where, X is the target force, x is the force data point and n is the number of samples.

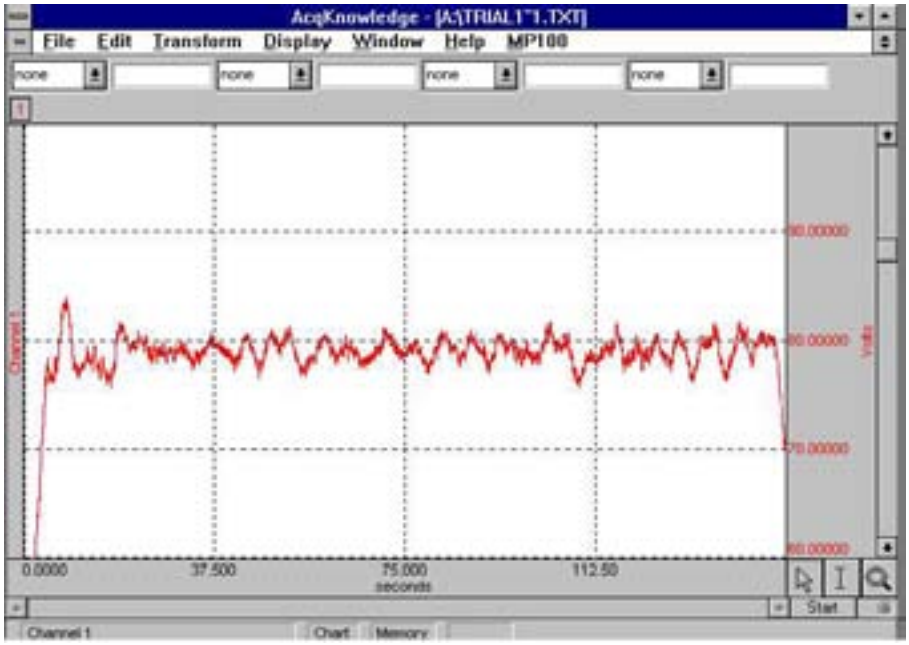


Figure 4.5 The Screen of the Isometric Neuromuscular Control Testing Software.

4.2.2.6 Isometric Muscle Endurance Capacity (IMEC)

IMEC was measured whilst the subject maintained a maximal isometric contraction for 30 seconds similar to that described by Bigland-Ritchie et al. (1983). The subjects were instructed to perform a maximal contraction without pacing themselves and were given standardised verbal encouragement throughout the test.

Due to the strenuous nature of the IMEC task the subjects only performed one test before and one after the drug on each test day. IMEC was defined as the integral of the force-time curve (impulse N.s,). The first 3 s of force data were excluded from this calculation due to inconsistent starts to this test.

4.2.3 EMG Data Collection and Analysis

The EMG signal from biceps brachii was collected during all MVIC-F, INC and IMEC tests. Bipolar Ag-AgCl surface electrodes were placed over the belly of biceps brachii in parallel with the muscle fibres and with an inter-electrode distance of 2 cm. A reference electrode was placed over the olecranon process of the elbow. The EMG signal was acquired by data acquisition hardware (BioPac, Santa Barbara, USA) with a set gain of 1000, and recorded by data acquisition software (Acknowledge, National Instruments, USA) sampling at 1000 Hz.

The EMG signal acquired during the MVIC-F, INC and IMEC tests, was analysed to identify any evidence of fatigue. The amplitude, median frequency and frequency bandwidth of the EMG were calculated. The typical myoelectric manifestations of fatigue during sustained isometric contractions include a decrease in EMG amplitude, median frequency and frequency bandwidth, the latter two variables indicating a shift in the muscle fibre type recruitment pattern (reviews by De Luca, 1984 and Vollestad, 1997).

Analysis of the EMG signal from the MVIC-F test was limited to the data between 0.5 and 2.5 s. The EMG signal from the IMEC tests were analysed in 3 x 3 s slices taken at the 3, 13 and 23 second time points. The raw EMG signal was band pass filtered between 10-500 Hz to remove any movement artefact and prevent aliasing errors. A power analysis of the filtered EMG was performed to obtain the spectral

characteristics of the EMG signal in the time domain. Power analysis of the filtered and rectified data was performed using customised software (LabView, National Instruments). The resulting signal was then high pass filtered at 10 Hz to smooth the frequency domain data. The median frequency of the fully processed EMG signal was defined as the frequency at which the area under the curve was divided into two equal halves. Bandwidth was defined as the frequency at which the amplitude of the frequency domain signal was at 50%. The peak amplitude of the EMG signal was determined after the filtered, detrended, rectified signal was passed through a root mean squared (RMS) function using a 100 ms sliding window. All analyses of EMG data were carried out using customised software (LabView, National Instruments).

4.2.4 Drug Administration

The serotonin receptor agonist (buspirone hydrochloride, Bristol Myers Squibb) or an indistinguishable placebo was administered in a blind fashion after the subject had completed the battery of neuromuscular function tests for the first time in each testing session. The drug or placebo was taken orally in tablet form (10 mg per tablet) with a glass of water. The dose of the drug was 0.375 mg/kg (body mass). An equivalent number of replica tablets made of lactose (Wille Laboratories, Australia), were administered as the placebo. The subject then rested for 60 min before repeating the battery of tests. Any adverse reactions or symptoms associated with the drug during this period and throughout testing were recorded.

4.2.5 Statistical Analysis

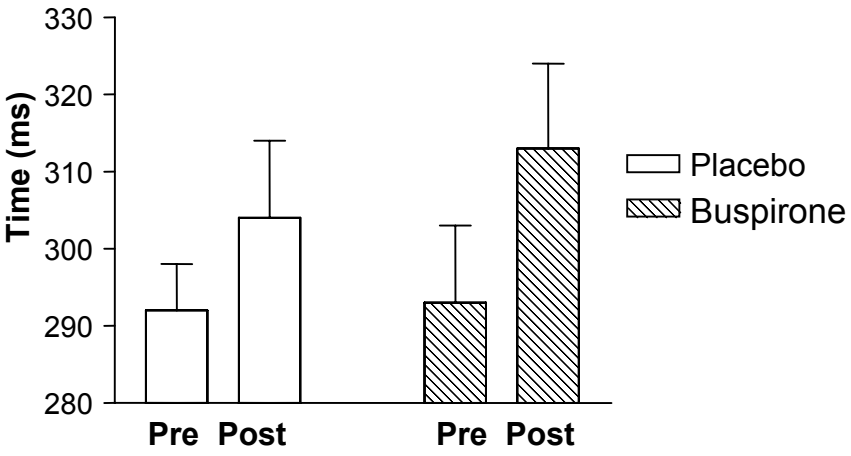
All data were checked for normality by the Kolmogorov-Smirnov test before applying a statistical test and were found to be normally distributed. Group means for Pre-Placebo and Pre- Drug, for each measure of neuromuscular function, was compared using a T-test to ensure that they were matched. The effect of the drug and placebo was calculated by determining the difference between Pre and Post scores. The significance of the effect of the drug was assessed by a T-test on the drug effect and the placebo effect on each measure of neuromuscular function. Data from the IMEC test, at three time points (3, 13 and 23 s), was analysed using a two way Repeated Measures ANOVA. Statistical significance was accepted at $p < 0.05$.

4.3 Results

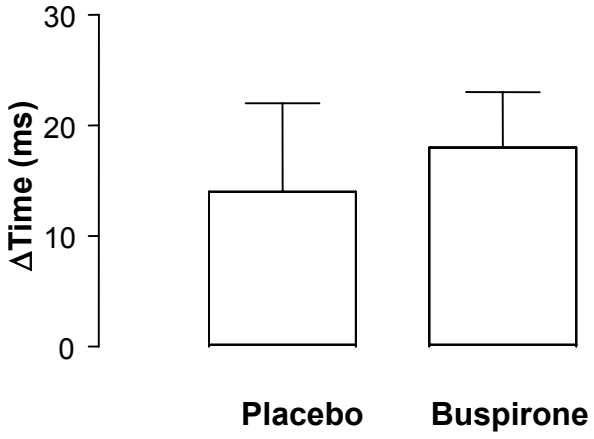
Some subjects reported some mild fatigue after receiving buspirone but did not experience any side effects that would adversely affect the results (eg. drowsiness, nausea or dizziness).

In this section each result is presented in a pair of figures. Figure A indicates the mean ± standard error of measurement of the raw scores, pre and post buspirone and the placebo. Figure B indicates the effect of buspirone and the placebo on the measure (ie. the change in the measure calculated as Post minus Pre).

Both buspirone and the placebo caused an increase in reaction time (293±10 vs. 313±11 and 292±6 vs. 304±10 ms respectively) and hence a decrease in performance. The increase in reaction time was slightly larger after buspirone, but the difference between the effect of buspirone and placebo was not significant (Figure 4.6B, p = 0.60)



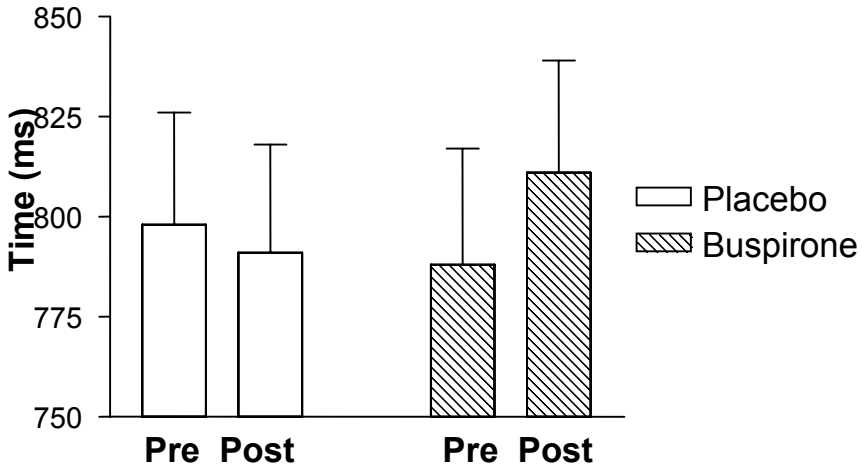
A



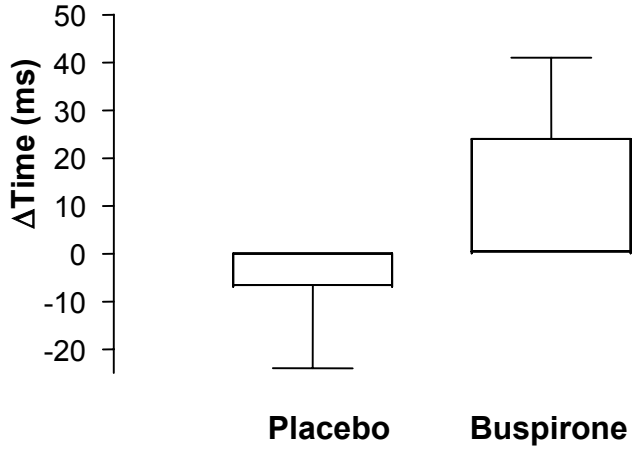
B

Figure 4.6 The Effect of Buspirone on Reaction Time. Figure A represents the group means for pre and post buspirone and placebo and figure B represents the mean change in (pre-post) in the measure for buspirone and placebo. There was no significant difference between the effects of buspirone and placebo on reaction time to a visual stimulus (p = 0.60).

The effect of buspirone and the placebo on hand eye coordination was measured as the response time to a computer generated coordination task. Hand eye coordination performance was slightly better after the placebo (798±28 vs. 791±27 ms) and slightly worse after buspirone (788±29 vs. 811±28 ms). Even though the effects of buspirone and placebo were opposite to each other, the overall effect was not statistically significantly different (Figure 4.7, p = 0.16).



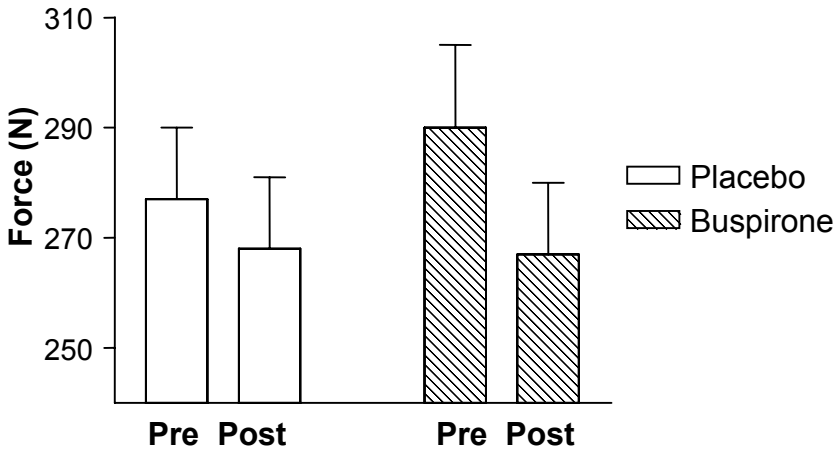
A



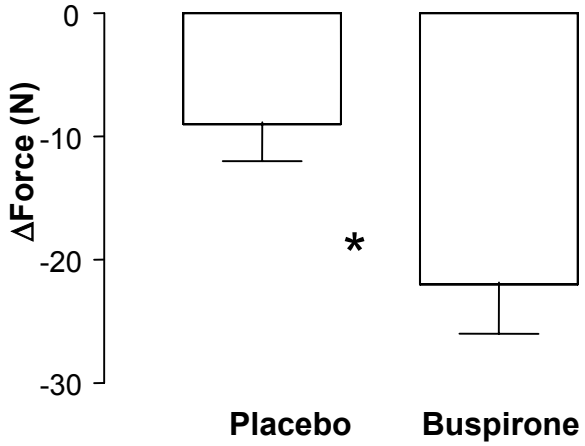
B

Figure 4.7 The Effect of Buspirone on Hand Eye Coordination. There was no significant difference between the effect (B) of buspirone and placebo on performance of a computer generated hand eye coordination task (p = 0.18).

There was a decrease in maximal voluntary isometric contractile force after administration of both buspirone and the placebo and the effect of busirone (-22.3±3.9 N) was significantly greater than the effect of the placebo (-8.7±2.9 N, p = 0.02). Mean MVIC-F measured before the placebo and buspirone appear to be different (276.0± 2.7 vs. 289.6±15.3 N respectively), but they were not statistically different (Figure 4.8A, p = 0.062). Nevertheless, the groups were not well matched on this measure, which weakens any conclusion about the effect of buspirone on MVIC-F.



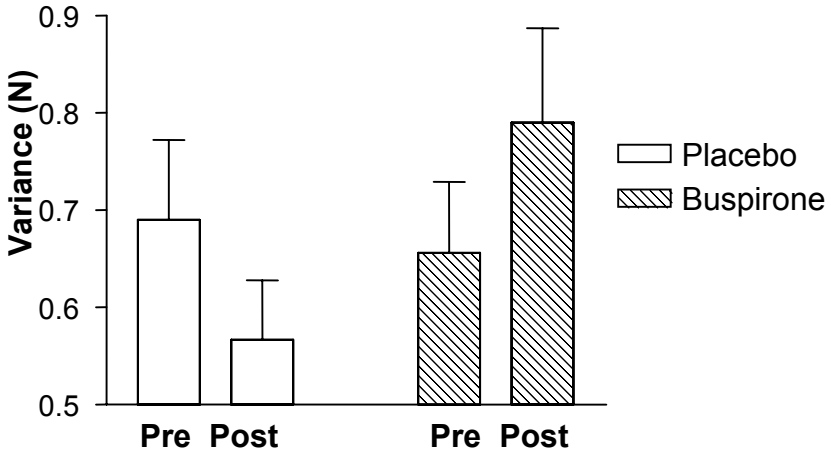
A



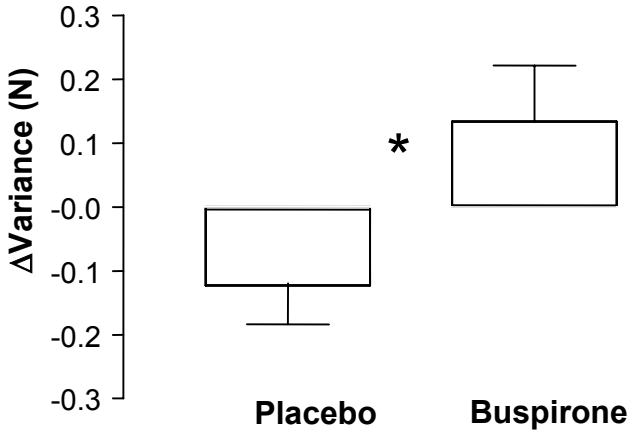
B

Figure 4.8 Effect of Buspirone on Maximum Voluntary Isometric Contractile Force. Buspirone caused a significant decrease in MVIC-F (B), compared to the placebo. * indicates a significant difference ($p < 0.05$).

Buspirone caused a decrease in isometric neuromuscular control (0.134 ± 0.087 N), while the placebo caused an increase (-0.123 ± 0.061 N). A decrease in INC was defined as an increase in the variance of contractile force and this was significantly different between the two treatments (Figure 4.9B, p = 0.04). This result indicates that buspirone reduced the ability to control the contractile force produced during a prolonged sub-maximal isometric contraction.



A



B

Figure 4.9 The Effect of the Buspirone on Isometric Neuromuscular Control. Buspirone caused a significant decrease in Isometric Neuromuscular control (B), measured as an increase in the variance of contractile force during a sub-maximal isometric contraction. * indicates a significant difference (p = 0.04).

Isometric muscular endurance capacity was not changed after placebo and decreased after buspirone. However buspirone caused a significantly greater decrease in IMEC (-407 ± 144 N.s, p = 0.004) than the effect of the placebo (Figure 4.10, -27 ± 132 N.s,).

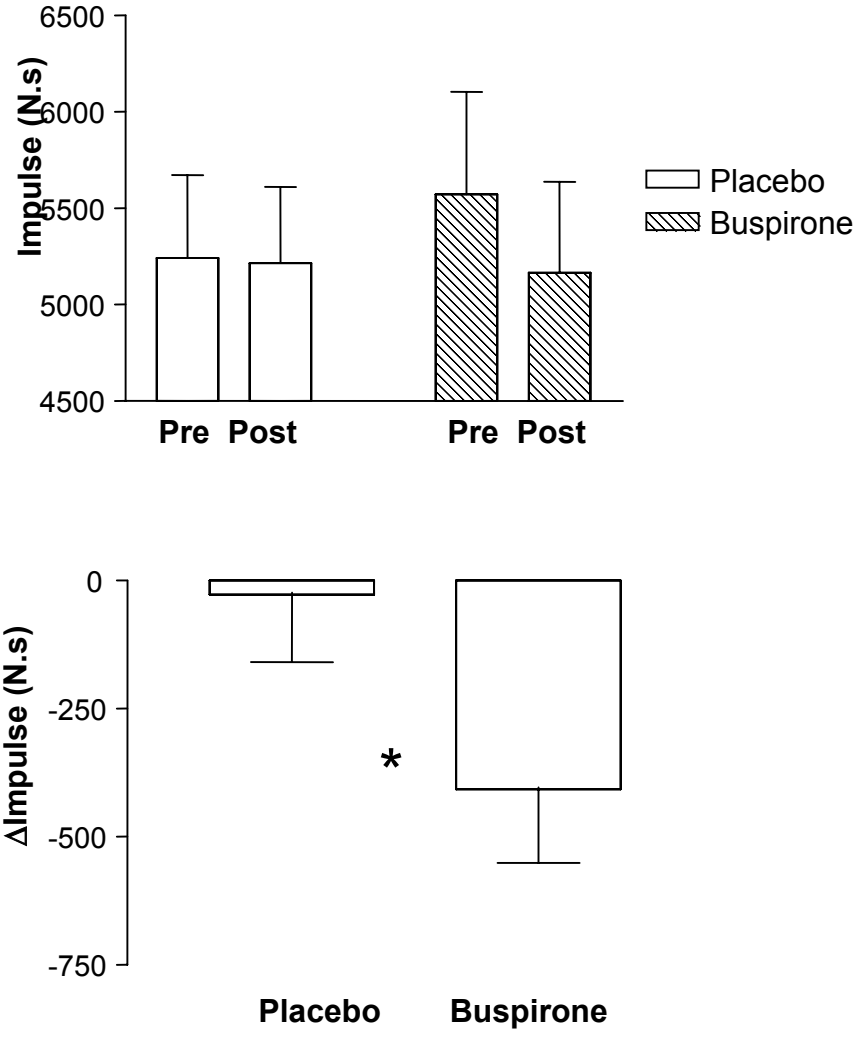
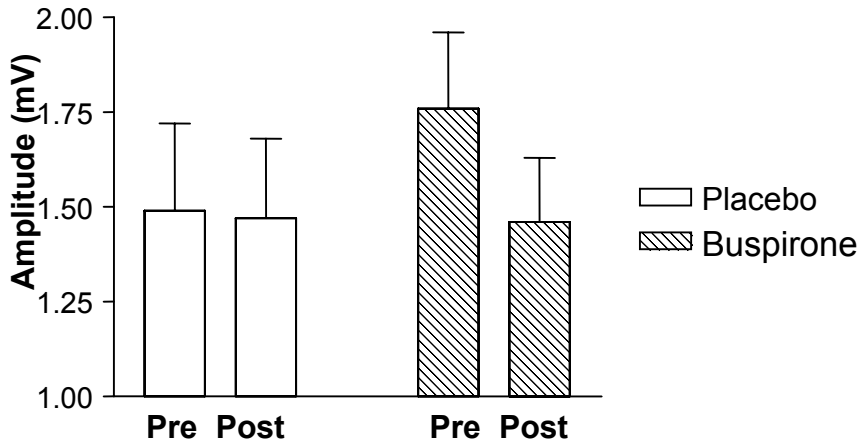
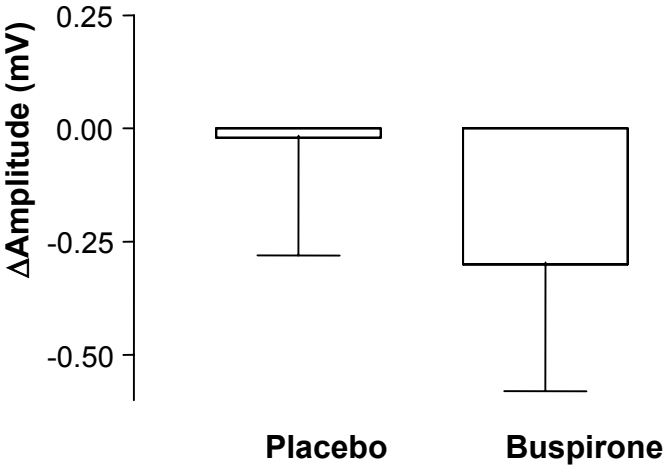


Figure 4.10 The Effect of Buspirone on Isometric Muscular Endurance Capacity. The decrease in IMEC after buspirone was significantly larger than the effect (B) of the placebo, indicating that buspirone causes a significant decrease in neuromuscular function. * indicates a significant difference (p = 0.004).

Spectral analysis of the EMG signal during the MVIC was performed in an attempt to identify any affect of the drug on motor cortex output. EMG amplitude was decreased after both buspirone and the placebo. While the decrease in amplitude appeared to be larger after buspirone it was not significantly different to the effect of the placebo (Figure 4.11B, p = 0.08). Mean EMG amplitude before the placebo and buspirone appear to be mismatched but were not statistically different (Figure 4.11A, p = 0.29).



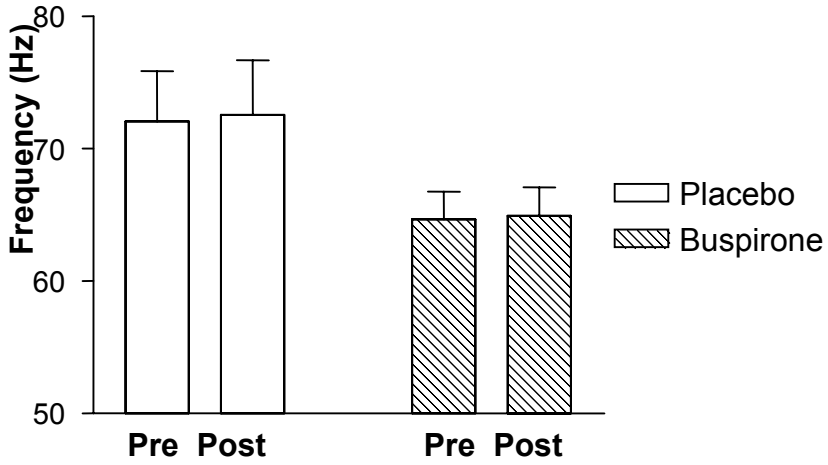
A



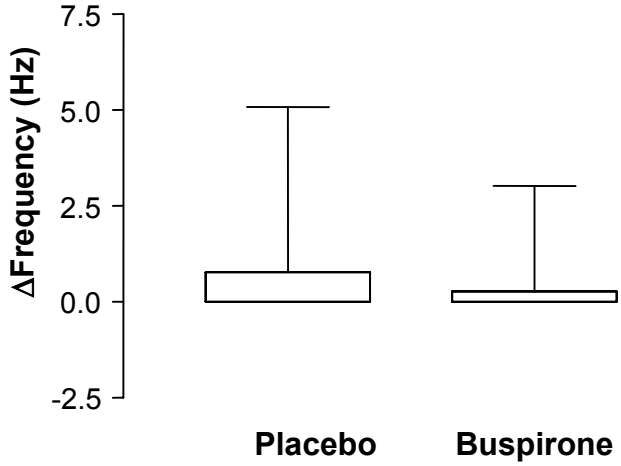
B

Figure 4.11 The Effect of Buspirone on EMG Amplitude During the MVIC-F Test. Although buspirone appeared to cause a decrease in EMG amplitude, the difference between the effect (B) of buspirone and the placebo was not significantly different (p = 0.08).

A decrease in the median EMG frequency during an MVIC would reflect a decreased ability to recruit fast twitch fibres, decreased conduction velocity and/or decreased motor unit discharge rate. There was no significant effect of buspirone on median EMG frequency during the MVIC-F test (p = 0.89). Mean median EMG frequency before the placebo and buspirone appear to be different but were not significantly different (Figure 4.12A, p = 0.13).



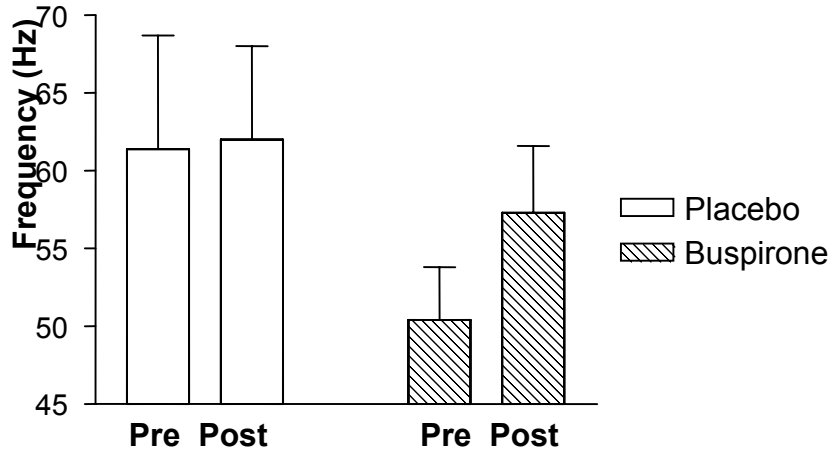
A



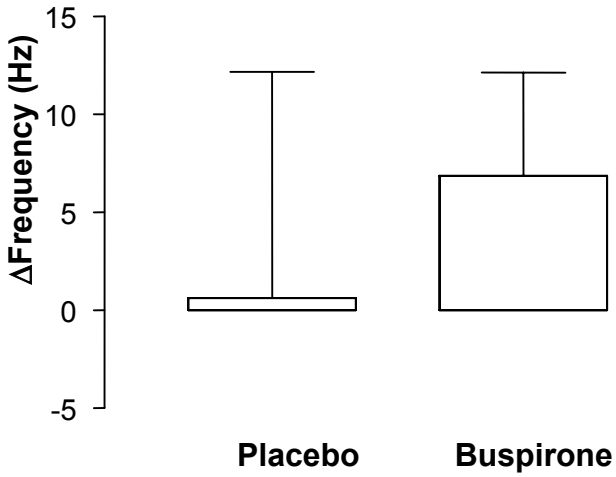
B

Figure 4.12 The Effect of Buspirone on Median EMG Frequency During the MVIC-F Test. There was no significant difference between the effect (B) of buspirone and the placebo on the median EMG frequency (p = 0.89).

The frequency bandwidth of the EMG signal during the MVIC was also assessed as a decrease in bandwidth often accompanies a decrease in median EMG frequency. Buspirone had no effect on the EMG frequency bandwidth during the MVIC-F test (p = 0.45). Mean EMG frequency bandwidth “pre” placebo and “pre” buspirone were also not significantly different (Figure 4.13A, p = 0.14).



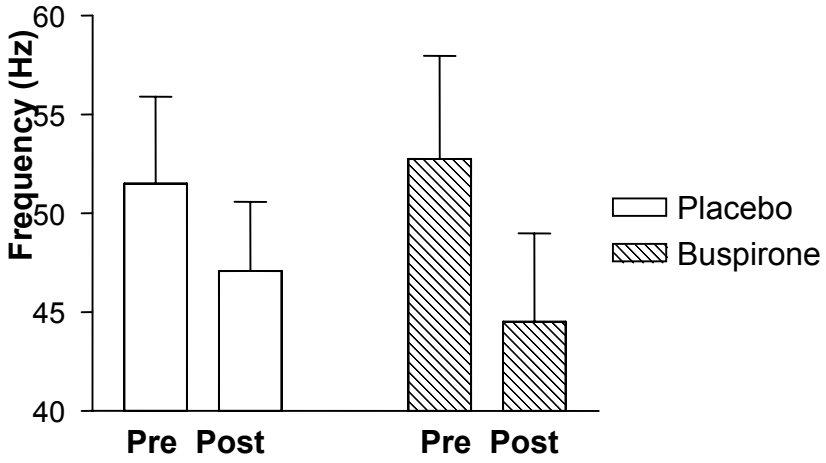
A



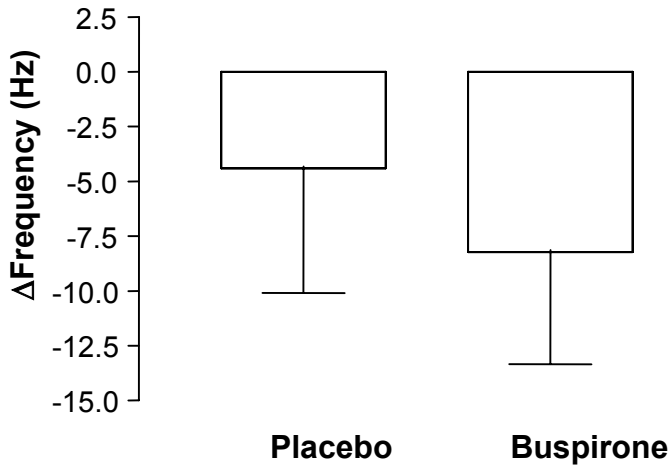
B

Figure 4.13 The Effect of Buspirone on EMG Frequency Bandwidth During the MVIC-F Test. There was no significant difference between the effec(B) of buspirone and placebo (p = 0.45).

During the INC tests, buspirone caused a non significant reduction in the EMG signal that was consistent with the onset of fatigue (ie. a decrease in median EMG frequency and frequency bandwidth). Both buspirone and the placebo caused a decrease in median frequency but there was no significant difference between these effects (Figure 4.14B, p = 0.65).



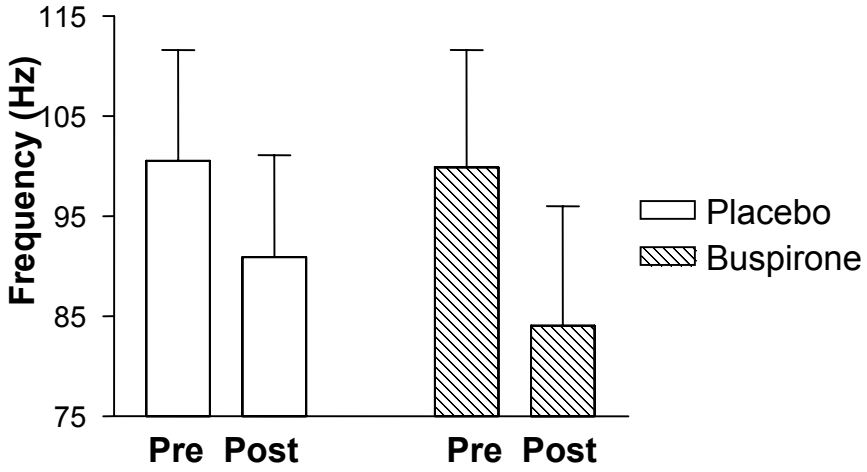
A



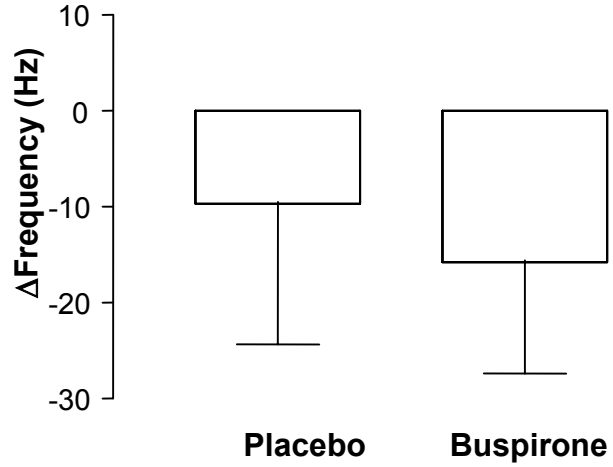
B

Figure 4.14 The Effect of Buspirone on the Median Frequency of EMG During the INC Test. There was no significant difference between the effect (B) of buspirone and the placebo (p = 0.65).

Both buspirone and the placebo caused a decrease in the frequency bandwidth during the INC test, however the effects of buspirone and the placebo were not significantly different (Figure 4.15B, p = 0.75).



A



B

Figure 4.15 The Effect of Buspirone on the EMG Bandwidth During the INC Trial. There was no significant difference between effect (B) of buspirone and the placebo on EMG bandwidth (p = 0.75).

The EMG signal from the IMEC test was analysed by comparing 3 second samples taken at 13 s and 23 s with a sample taken at 3 s. The EMG signal was analysed for changes in amplitude, median frequency and frequency bandwidth in order to identify any changes in motor cortex output that were consistent with fatigue. There was a tendency for EMG amplitude to decline post buspirone while amplitude tended to increase post placebo (Figure 4.16). However, there was no significant differences between the response to buspirone and the placebo at 13 s ($p = 0.40$) or 23 s ($p = 0.50$).

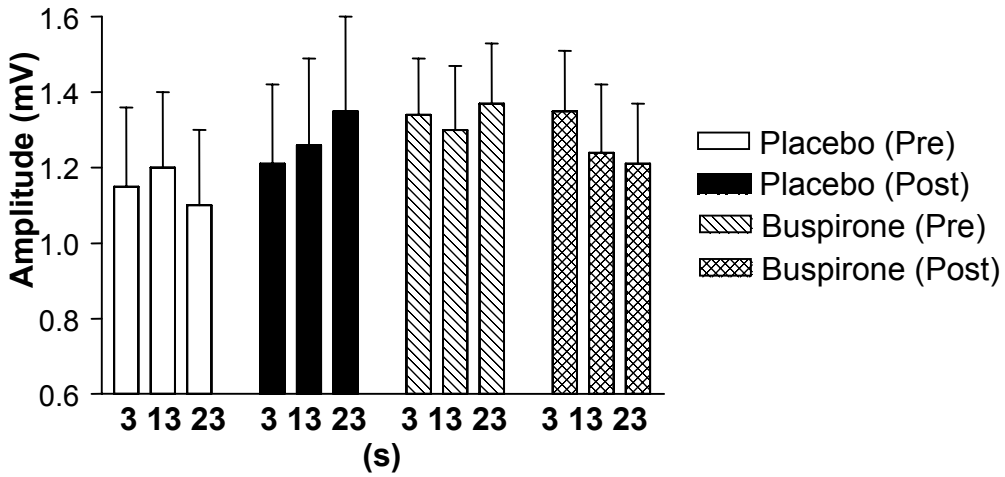


Figure 4.16 Changes in EMG Amplitude During the IMEC Test. Amplitude is expressed relative to the first measure at 3 s. There was no significant difference between the effect of buspirone and the placebo on EMG amplitude ($p = 0.50$).

Median EMG frequency tended to decrease throughout the IMEC test after both buspirone and the placebo (Figure 4.17). There was also no significant difference between the effect of buspirone or placebo on median EMG frequency at 13 s ($p = 0.08$) or 23 s ($p = 0.10$).

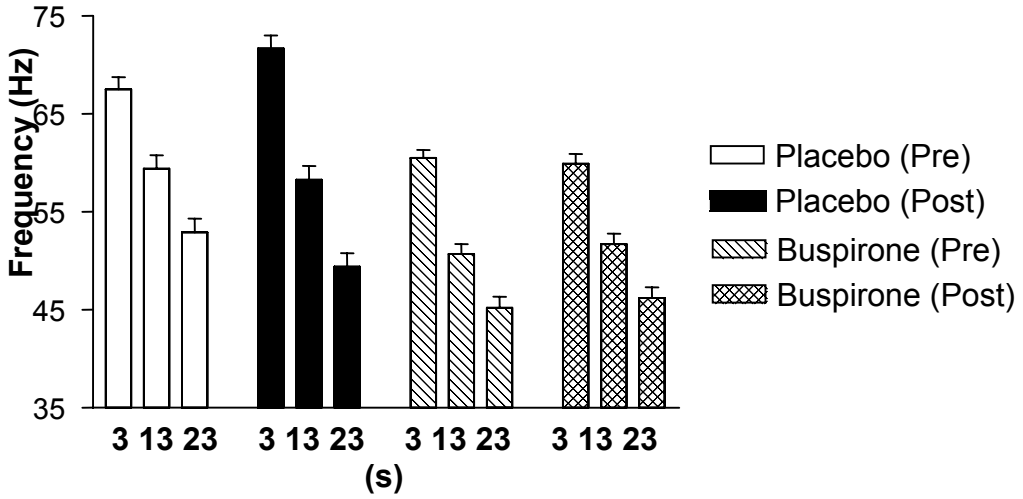


Figure 4.17 Changes in Median EMG Frequency During the IMEC Test. Median EMG frequency is expressed relative to the first measure at 3 s. There was no significant difference between the effect of buspirone and the placebo on Median frequency ($p = 0.10$).

There was a significantly different effect of buspirone compared to the placebo, on EMG frequency bandwidth at 13 s (p = 0.04) but not at 23 s (p = 0.11). EMG frequency bandwidth was lower “post” placebo when compared to “post” buspirone (Figure 4.18). This finding may be related to the difference the initial bandwidth (at 3 seconds) between pre and post placebo rather not a genuine physiological effect.

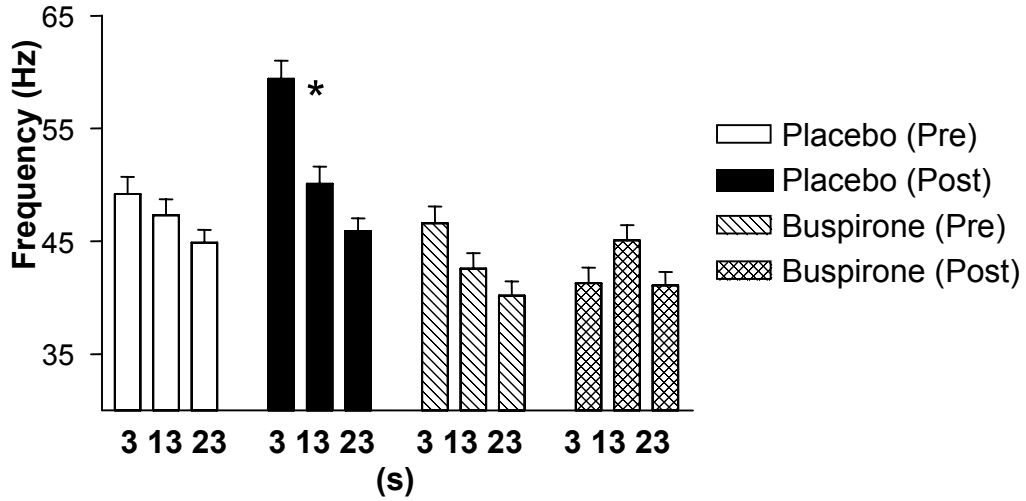


Figure 4.18 Changes in EMG Bandwidth During the IMEC Test. EMG frequency bandwidth is expressed relative to the first measure at 3 s. There was a significant decrease in frequency bandwidth after the placebo at 13 s (p = 0.04).

4.4 Discussion

4.4.1 Design Considerations

The purpose of this study was to examine changes in muscle function associated with the effect of a serotonin agonist (buspirone), therefore it was important that the tests themselves did not cause fatigue in the subjects. The order and the duration of tasks and rest periods were carefully considered. The most strenuous tests, MVIC-F and IMEC were completed last to avoid any residual fatigue affecting subsequent tests involving fine motor control. However, the MVIC-F tests had to occur before the INC test to determine the target force (30% of MVIC-F) for that test.

The INC test was designed to examine changes in the ability of the central nervous system (CNS) to control a sustained sub-maximal contraction. It was important to attempt to isolate the effect of local muscle fatigue from this test (eg. the accumulation of metabolites), as it would be interpreted as fatigue in the CNS. A pilot study was conducted to determine the maximum duration of three consecutive INC tests that showed no evidence of fatigue. Thirty second tests were determined to be too long and 15 s tests showed no evidence of residual fatigue after three tests. Additionally, the relative target force of this test was chosen as 30% of MVIC-F, which was based on a similar test protocol previously reported (Krogh-Lund and Jorgensen, 1993). Further tests were conducted to determine the ideal scale of the force graph that was provided to the subject for real time feedback. A large scale made the initial increase in force (up to the target force) easy but provided little detail on small changes in force once at the target force. A smaller scale offers the opposite features. A scale that shows $\pm$20% of the target force was found to be the most appropriate and provided the best INC performance (Figure 4.5).

The IMEC test was designed to assess the ability of the subject to sustain a maximal contraction over a relatively long duration. Initial tests indicated that 30 s was long enough to elicit a substantial decrease in force production. Three 30 s tests were found to cause too much residual fatigue and hence, were likely to effect subsequent test results. Additionally, there was no added benefit to performing a second test, in terms of detection of fatigue caused by the drug, by performing the second two tests.

The delay of testing after the dose of the agonist (dose delay) was also considered. It was the goal of this experiment to examine the effects of the drug at the time point of maximum effect. The researcher and a colleague participated in a pilot study, where the test protocol was administered every 60 minutes for 3 hours after a dose of the drug. The largest decreases in neuromuscular function occurred after 60 min and 120 min. A 60 min delay was chosen, as it minimised the duration of each session.

One of the purposes of the present work was to examine the effect of a serotonin agonist on maximum voluntary contractile force (MVIC-F). MVIC-F may decrease because of sub optimal motor cortex output (central fatigue) or local muscle fatigue. The twitch interpolation technique, involving artificial tetanic stimulation, has been used to identify the origin of fatigue as being central or peripheral (Gandevia and McKenzie, 1988). The presence of this technique would have provided additional confidence regarding the central nature of the fatigue caused by the drug. However, there are several features of the method employed here that may obviate the need for the twitch interpolation technique. Firstly, any decrease in motor cortex output, due to central fatigue during the MVIC, would have been evident in changes in EMG amplitude, median frequency and bandwidth. The present study recorded and analysed these indices of fatigue. Secondly, it is unlikely that local muscle fatigue

had a substantial effect on MVIC-F, given that the contractions were few and short (3 x 3 s). Local muscle fatigue usually occurs due to the cumulative effects of repeated or sustained contractions and becomes more pronounced over time. However, there was no trend of decreasing force from the first to the third MVIC-F in the present data. In support of the claim that local muscle fatigue probably didn't affect MVIC-F is the conclusion of Gandevia et al. (1995), that well motivated subjects can achieve their maximal evocable force (ie. no local muscle fatigue) in their initial attempts at an MVIC. Lastly, if local muscle fatigue did affect MVIC-F then it would have been corrected for by the cross over design of the present study and the type of comparison made. Any local muscle fatigue in the post drug MVIC-F test should also have been present in the post placebo MVIC-F test. As the subjects served as there own controls the effect of the drug could be directly compared with the effect of the placebo. Therefore any difference in the group means is due to the central effects of the drug.

An important limitation of the design of the present work is the lack of specificity of buspirone. The 5-HT1a receptor is located both postsynaptic to serotonin neurones and on the neurones themselves (ie. presynaptic, Barnes and Sharpe, 1999). Although it appears that the distribution of presynaptic receptors varies throughout the brain. A review by Hoyer et al. (1994) suggests that the density of presynaptic receptors in the forebrain is low and that hippocampal 5-HT receptors are essentially postsynaptic. Nevertheless, there is some confusion in the literature about whether buspirone is an agonist for pre or postsynaptic 5-HT1a receptors and hence, whether it stimulates or inhibits serotonergic activity. An early review of the pharmacology of buspirone suggested that it is an agonist for presynaptic receptors and that it inhibits serotonergic activity (Eison and Temple, 1986). Vandermaelen et al. (1986) demonstrated that buspirone decreases the spontaneous firing of serotonergic neurones in the dorsal raphe neuclei in the rat brain. In contrast to this

finding, was that of Hjorth and Carlsson (1982) who described buspirone as a “5-HT mimetic”, as brain 5-HT levels were unaltered after its administration and therefore it was probably not active presynaptically. In addition, there are numerous studies which demonstrate that buspirone and other agonists activate post synaptic hypothalamic receptors, evidenced by the secretion of several hormones (eg. prolactin, cortisol and growth hormone; Coccaro et al., 1990; Cowen et al., 1990). Finally, in the recent attempts to clarify how much of the neuroendocrine response to buspirone is mediated by serotonergic receptors, antagonists for post synaptic serotonin receptors (eg. pindolol) have been shown to attenuate the prolactin response to buspirone (review by Bridge et al., 2001). These findings suggest that buspirone is an agonist for post synaptic receptors. The most recent evidence on this issue is from McAllister-Williams and Massey (2003), who reported a decrease in the median frequency of brain waves (electroencephalography, EEG) in human subjects after 30 mg of buspirone. While the decreased median frequency suggests decreased firing rate of neurones, it not clear whether the neurones were serotonergic or not, as the EEG was not localised to a particular part of the brain. If they were serotonergic neurones then this would implicate a presynaptic effect of buspirone, conversely buspirone may have increased the degree of inhibition a serotonergic neurone was exerting on another type of neurone (eg. a monoaminergic spinal motoneurone).

In conclusion, the action of buspirone is likely to vary according to the dose and the brain region under examination, given the varied distribution of receptors (Barnes and Sharpe, 1999). The dose used in the present study (25-30 mg) has been shown previously to cause increase in prolactin secretion suggesting that buspirone acted postsynaptically and inhibited serotonergic function (Seppala et al., 1987; Meltzer at al., 1994).

A further limitation of the specificity of buspirone is the possibility that some of its effects may be partly mediated by receptor sub types other than 5-HT1a or even dopamine (D2) receptors. The possibilities of peripheral effects of the drug were also considered (eg. effects on peripheral motoneurones and nociception), however the only serotonin receptors that are known to exist in the peripheral nervous system are 5-HT2a, b and 5-HT3 sub types, which are unlikely to have affected the results in the present study (Hoyer et al., 1994). Furthermore, the changes reported here in EMG amplitude are not likely to have been caused by any peripheral effects of the drug, as there are no known serotonin receptors that innervate motor neurones in the peripheral nervous system (Hoyer et al., 1994). Nevertheless, buspirone's effect on dopaminergic function may be involved in changes in neuromuscular function reported here.

Finally, the way the data were analysed and presented here was considered carefully. Before the study was conducted it was anticipated that there would be some day-to-day variability in some of the measures used here. For example, it would certainly not have been possible to compare EMG signals from day to day as the impedance of electrode-skin contact changes slightly as each new electrode is applied to the skin. Furthermore, it was also possible that this day-to-day variability might have been larger than the effect of the drug on each measure of neuromuscular function. Therefore the data analysis and presentation was designed so that the day-to-day variability was prevented from obscuring any effect of the drug or placebo on each measure of neuromuscular function. This was achieved by only comparing post drug scores with the pre drug scores collected on the same day and separately, post placebo with pre placebo scores. The alternate method of simply combining pre placebo and pre drug scores into one "base line" group for comparison with both "post" scores, would have introduced this day-to-day variability and reduced the sensitivity of the analysis.

4.4.2 Discussion of Results

The present investigation provides evidence that the serotonin receptor agonist, buspirone, can cause a significant reduction in neuromuscular function during isometric muscle contractions. Buspirone reduced the ability to control a sustained submaximal contraction (INC), the total cumulative force applied over a sustained maximal contraction (IMEC) and reduced maximum isometric contractile force (MVIC-F). These changes are consistent with exercise induced fatigue and would be likely to decrease performance during dynamic exercise.

An increase in brain serotonin is known to cause fatigue (Davis & Bailey, 1997; Chaouloff, 1997) and it was hypothesised in the present study, that this might also be evident as a decrease in reaction time and hand eye coordination. There are reports of both an increase and a decrease in reaction time after buspirone administration (review by O'Hanlon, 1991). There are almost no similar studies that measured the effect of buspirone on hand eye coordination. The majority of previous research in this area was intended to explore the effects of buspirone on driving safety and oculomotor coordination was tested rather than hand eye coordination. The exception is a study by Moskowitz and Smiley (1982), who found no decrement in a driving performance task, which indirectly assessed hand eye coordination, after buspirone administration. The serotonin agonist used in the present study did not significantly affect RT or HEC. There were however qualitative decrements in both of these variables that may become significant at higher doses of the drug. Until all of the inhibitory effects of serotonergic neurones on motoneurones are fully described, it will be difficult to predict the effect of serotonin agonists and central fatigue on neuromuscular function.

Interpretation of the effect of the drug on MVIC-F is somewhat problematic because the MVIC-F scores pre drug and pre placebo do not appear to be the same. It is difficult to explain why there was so much variation between these two groups. If the daily variation in this measure is normally large, then one would expect to see it the "post" data as well, but it was not evident. It is not possible that a systematic error biased the "pre" measurements for the drug treatment, because the order of treatments was randomised and the test protocol was adhered to for all testing. Despite this limitation, the author feels that as there was no difference statistically between the "pre" results and the effect of the drug was significantly larger than the effect of the placebo, buspirone did cause a significant decrease in MVIC-F. In contrast to this finding are two previous reports that a simulated increase in brain serotonin and found no significant change in grip strength (Schaffer and Klausnitzer, 1988; Cunliffe et al., 1998). However, comparison with these reports is difficult because in the first report the dose of buspirone was different (5 vs. 25-30 mg) and in the second report tryptophan was used to increase brain serotonin.

The decrease in MVIC-F after the administration of the drug in the present study probably indicates a reduction in the ability of the central nervous system to generate adequate neural drive for the elbow flexors. The decrease in MVIC-F in the present study may be due to spatial and/or temporal changes in the EMG signal (ie. the number of motor units activated and/or the firing rate of motoneurones, Enoka, 1994). This contention is supported by two prior reports of a decrease in the firing rate of spinal motoneurones in rats after treatment with a serotonin agonist (Beato and Nistri, 1998; Spampinato et al., 1985). If a decreased firing rate of motoneurones also occurred in humans, it might be evident in the frequency characteristics of the EMG signal collected during voluntary muscle contraction. The results of the present study offer some support for this, as there was a decrease in EMG amplitude during the MVIC-F tests, although this decrease did not reach

statistical significance ($p = 0.08$). There were no significant differences in median frequency or bandwidth of the EMG signal between the drug and placebo tests in the present study.

The INC test was included to examine the hypothesis that central fatigue would adversely affect the ability to control contractile force during an isometric contraction. The statistical variability of the isometric contractile force was used as an indicator of control. The drug caused an increase in variability of force, which indicates decreased control. This may have consequences for locomotion and performance, since the ability to make coordinated movements depends partly on well controlled muscle contractions. However, only force control during an isometric contraction was examined, not during concentric or eccentric contractions, which remains a limitation of the present work.

There was a significant decrease in impulse force during the IMEC test as a result of the drug. This finding contradicts a report by Cunliffe et al. (1998) who showed an increase in wrist ergometric performance after tryptophan supplementation. Although these researchers concluded that experimental errors rather than a genuine ergogenic effect were responsible for the increase. Unlike the MVIC-F test in the present study, the decrease in impulse force was not due to a reduction in EMG amplitude, nor was there any difference in median frequency between treatments. However, the placebo caused a paradoxical decrease in frequency bandwidth from the 3 s to the 13 s sample point. As there was no change in median EMG frequency at 13 s, the decrease in bandwidth probably did not have a significant effect upon contractile force. The failure to identify any change in EMG measures of fatigue contradicts what might be expected to occur with a decrease in impulse force. It is concluded that the changes in EMG amplitude were functionally important, but too small to be statistically significant.

The possibility that the neuromuscular changes reported here, may be due to the sedative effect of the drug was considered. Buspirone has been used as a replacement for older drugs (eg. benzodiazepines) that have known sedative and hypnotic effects. However, in a review of the effects of buspirone on human performance, O'Hanlon (1991) concludes that "buspirone possesses a very low sedative potential". The experiences of the present author would lead to the same conclusion. Only a couple of the subjects in the present study reported transient periods of tiredness. When this occurred testing was delayed momentarily until the subject was asymptomatic. Therefore it is unlikely that sedation was responsible for the change in neuromuscular function reported here.

In conclusion, the detrimental effects of central fatigue on endurance exercise performance reported previously (Marvin et al., 1997; Wilson and Maughan, 1992) are likely to be due to a combination of several factors. An increase in brain serotonin is known to exert several effects including decreased cardiac output (Bagdy et al., 1989) and an increase in perceived exertion (Marvin et al., 1997). Either of these factors individually could be responsible for the reduction in performance during endurance type exercise. The present work demonstrates that central fatigue may also reduce neuromuscular function during isometric contractions. In addition, because this type of fatigue is due to suboptimal output from the motor cortex, similar decrements in neuromuscular function during concentric contractions may also occur under the influence of central fatigue. However, this hypothesis remains to be tested. The possibility that the dopaminergic effects of buspirone were involved in the results reported here cannot be discounted. The strength of the conclusions of this study will be enhanced when more selective serotonin receptor agonist drugs, suitable for use in humans, become available.

5.0 General Discussion

The aim of this thesis was to identify adaptations in the central serotonergic systems that occur as a result of exercise training and to describe the effect of a serotonin agonist on neuromuscular function. It was hypothesised that exercise training in experimental animals and human subjects would cause a measurable decrease in serotonin receptor sensitivity. In addition it was hypothesised that the simulated increase in brain serotonin via the administration of a serotonin agonist would cause a decrease in neuromuscular function that was analogous to fatigue.

The results presented in Chapter 2 suggest that exercise training in rats causes an adaptive decrease in serotonin receptor sensitivity, measured as a decrease in the response to the 5-HT1a agonist m-CPP. There was a decrease in receptor sensitivity that was sustained from weeks 3 to 6 of training. During this period the adaptation appeared to plateau indicating that a limit of adaptation had been achieved or that the training stimulus had become inadequate. This is the first evidence of a training induced adaptation of a post synaptic serotonin receptor in the CNS. Previously, treadmill running for 3 and 5 weeks had been shown to decrease 5-HT1b (Seguin et al., 1998) and 5-HT1a (Dey, 1994) pre synaptic receptor sensitivity in rats. The effect of training on post synaptic receptors was less clear with reports of no change after 4 days of running (Chaouloff, 1994) and a non significant decrease after 4 weeks of swimming in rats (Dey, 1994). This finding provides support for the possibility that training adaptations take place in the brain and may affect exercise performance in mammals. The relationship between receptor sensitivity and endurance performance also suggests that central fatigue may be an important determinant of endurance performance in rats and possibly other mammals.

After observing these changes in rats, evidence of a similar adaptation in human subjects was sought in the experiments described in Chapter 3. There was no change in the neuroendocrine response to the serotonin agonist buspirone, as a result of 9 weeks of cycle training and therefore probably no change in receptor sensitivity. This finding contradicts the findings of Chapter 2 and those of previous reports that found a difference in receptor sensitivity between trained and untrained subjects (Jakeman et al., 1994; Broocks et al., 1998). The differing responses of the rats and humans to a serotonin receptor agonist in the present study may reflect the inadequacy of the training stimulus described in chapter three. In other words, there is likely to be a species related difference in the response to exercise training. Although it is problematic to compare the relative intensity of the training regimens applied to rats and humans, it appears that central serotonin receptors in rats require a shorter period to adapt than in humans. Therefore there is evidence that serotonin receptor sensitivity can change, but whether exercise training can stimulate this change in humans remains to been confirmed.

After the findings of Chapter 3 were submitted for publication, Broocks et al. (2001) published the results of a similar study. Twelve untrained subjects trained three times per week for ten weeks. The neuroendocrine response to agonists of 5-HT1a and 5-HT2c receptors was used to identify changes in serotonin receptor sensitivity, before and after training. These authors reported a decrease in 5-HT2c receptor sensitivity but no change in 5-HT1a receptor sensitivity, the latter reflecting the findings of Chapter 3 in the present work. However their data analysis was unusual and may have biased their findings. Rather than comparing the neuroendocrine response to an agonist before and after training, these authors compared the response to the agonist with the response to a placebo. There was a significant difference between the prolactin response to the 5-HT1c agonist (m-CPP) and the placebo before training (304.0 ± 245 vs. 62.7 ± 78 μU/mL), but no difference after

training (285.8 ± 319 vs. 77.2 ± 76 μU/mL). As there was no difference between the prolactin response to the serotonin agonist and the placebo after training, the authors concluded that there must have been a decrease in receptor sensitivity. However, there was probably no significant difference between the prolactin response to the serotonin agonist before and after training (304.0 ± 245 vs. 285.8 ± 319 μU/mL). In addition, the authors have ignored much of the detail in the neuroendocrine response by choosing to compare mean maximum increase in prolactin, rather than the area under the response curve, or by comparing a series of values taken at set time points after the initial dose. These authors also assessed the cortisol response to the serotonin agonists in the same way to strengthen their conclusion.

The report by Broocks et al. (2001) highlights the difficulty of using neuroendocrine challenge tests to probe the sensitivity of central serotonin receptors and does not provide clear evidence of a decrease in 5HT1c receptor sensitivity. Nevertheless, if further research in this area does provide clear evidence that exercise training in human subjects does lead to decreased receptor sensitivity, then there may be important implications for the treatment of disorders involving serotonergic dysfunction. For example, increased serotonin receptor sensitivity has been reported in those suffering from depression (Dey, 1994) and chronic fatigue syndrome (Bakheit et al., 1992). Exercise training may attenuate the increased receptor sensitivity and serve as an adjunct therapy for depression and chronic fatigue syndrome.

The results presented in Chapter 4 provide evidence that the serotonin receptor agonist, buspirone, causes a decrease in several objective measures of neuromuscular function. This finding partly explains the way in which serotonin receptor agonists employed by other researchers brings about a reduction in

exercise performance. In the past it has been implied that central fatigue depresses neuromuscular function, but there was little direct evidence to support this assumption. Further research is warranted to describe the effect of serotonin receptor agonists on neuromuscular function during other types of muscle contractions. However, similar decrements are likely to occur since the site of fatigue is the CNS, which is involved in all types of muscle contractions.

There may be practical implications of the findings described in Chapter 4 for sports people and possibly those who take serotonin agonists for therapeutic reasons. Presently, central fatigue has only been considered to effect performance during long duration exercise like running. However, the present results indicate that central fatigue may be important to sports people who participate in events requiring intermittent tasks requiring near maximal muscle contractions (field events, wrestling, etc.) rather than rhythmic submaximal contractions which are typical of running or swimming. However, changes in plasma Branch Chain Amino Acids and Free Fatty Acids are usually required to initiate an increase in brain tryptophan, brain serotonin and hence cause central fatigue. This process requires time to occur, perhaps more than 30 minutes, which is why central fatigue has been examined during relatively long duration events (eg. the marathon, Blomstrand et al., 1988). Nevertheless, athletes who compete in short duration events often exercise for a longer period prior to the event in a warmup and/or a series of qualifying heats. This period of activity may be sufficiently long and intense to stimulate the requisite changes in plasma Branch Chain Amino Acids and Free Fatty Acids.

Serotonin agonists taken for therapeutic reasons may also cause central fatigue and effect normal motor function. For example, buspirone is prescribed as an anxiolytic in relatively low doses (15-60mg/day). Chronic administration may cause a

decrease in neuromuscular function and effect normal daily functioning. Although, this effect may be mitigated by increased tolerance to the drug by down regulation of serotonin receptors.

The present work provides two pieces of evidence that serotonin antagonists might improve endurance performance. The relationship between receptor sensitivity and endurance performance in rats highlights the importance of central fatigue as a limiting factor of endurance performance, albeit in experimental animals. In addition, the results of Chapter 4 demonstrated that buspirone decreases neuromuscular function. The possibility that a serotonin antagonist might increase endurance performance has been examined twice (Meeusen et al., 1997; Pannier et al., 1995) and neither study found an increase in performance. However, the authors of the latter report conceded that the side effects of the drug they employed may have masked its positive effects. Therefore a carefully chosen antagonist that discretely selects for 5-HT1a receptors and causes few side effects is likely to extend endurance performance in humans. Presently there is only one drug that affects serotonergic function on the list of banned substances published by the International Olympic Committee. Fenfluramine has amphetamine like properties as well as being used to reduce appetite. New candidate drugs that may offer performance enhancement include spiperone, methiothepin and ergotamine, which are all agonists for 5-HT1a receptors, the last being a partial agonist for 1b and 1d receptors as well. While these drugs do have a legitimate therapeutic use, they should be added to the IOC's list of banned substances because of their ergogenic potential.

5.1 Conclusions

Exercise training in rats causes an increase in exercise performance and an increase in the tolerance to a serotonin agonist (m-chlorophenylpiperazine). This increased tolerance probably reflects the desensitisation of 5-HT receptors in the CNS, although other receptor types may be involved (eg. dopaminergic). Sensitivity to a serotonin receptor agonist was somewhat predictive of endurance exercise performance. This finding lends weight to the possibility that desensitisation of central serotonin receptors allows trained animals to better tolerate the increases in brain serotonin induced by exercise. In addition the capacity of an organism to desensitise central serotonin receptors may be a determinant of endurance exercise performance.

Exercise training in human subjects did not decrease serotonin receptor sensitivity, despite increases in aerobic power and endurance capacity. However, there were limitations in the technique used and there may have been undetected changes in other serotonin or dopaminergic receptor subtypes. In addition, if desensitisation of receptors does occur as result of exercise training, it occurs at a slower rate than peripheral adaptations that enhance endurance performance (eg. increased plasma volume, skeletal muscle hypertrophy, etc.). Highly selective serotonin agonists that are suitable for human experimentation are required to further assess the changes in serotonin receptor subtypes in response to exercise training.

The administration of the serotonin agonist, buspirone, decreases several measures of neuromuscular muscle function in man. The decrease in maximum isometric contractile force was accompanied by a decrease in EMG amplitude that was marginally significant. The decrease in neuromuscular function is likely to be mediated by, although not exclusively, 5-HT inhibitory neurones in the

hypothalamus. These results help to explain how an increase in brain serotonin causes fatigue and a decrease in exercise performance in experimental animals and human subjects.

5.2 Future Research Directions

The present understanding of central fatigue is largely based upon our understanding of the distribution and function of serotonergic neurones throughout the CNS. If the nature of central fatigue is to be fully elucidated, ongoing development of highly specific agonists for all serotonin receptors is required. New technology such as Nuclear Magnetic Resonance Spectroscopy may also offer opportunities to directly assess serotonergic function in the intact human brain.

As a change in receptor sensitivity in trained rats was detected, further examination of this process is warranted. The use of autoradiography to visualise central serotonin receptors would determine whether the change in receptor sensitivity was due to a change in receptor population size or receptor function. Simultaneous examination of changes in the concentration of brain tryptophan, serotonin and 5-hydroxyindoleacetic acid in several brain regions, is required to validate the two reports on this topic. Future investigations of changes in receptor sensitivity in humans should take advantage of a recently published neuroendocrine challenge test that is more accurate than the technique used in the present work. Bridge et al., (2001) describe the use of a serotonin (5-HT1a) antagonist (pindolol) in concert with Buspirone to more accurately determine the sensitivity of serotonin receptors.

The methodology employed in Chapter 4 simulated central fatigue by the administration of a serotonin agonist, which is not the same as an exercise induced

increase in endogenous serotonin in the brain. The dose and receptor selectivity of the drug would have undoubtedly caused different effects that may present slightly different neuromuscular effects than exercise induced central fatigue. The ideal research design would be to replicate the exercise induced increase in brain serotonin in a controlled manner, employing a standardised exercise protocol. However, the difficulty in bringing about a reproducible “natural” form of central fatigue is in isolating peripheral factors of fatigue from the effects of central fatigue. If this were possible, the fatiguing effects of an exercise induced increase in brain serotonin could be described more accurately. Presently there are few serotonin receptor agonists available for use in human subjects, and their precise affinity for receptors is not well known. The validity of the results of the present and future work would be greatly enhanced if more selective agonists were available.

Serotonin receptor antagonists have been shown to improve performance in rats but not humans. The absence of any positive effect in humans is probably due to the lack of specificity of the antagonist used and its subsequent side effects. An alternate strategy to delay or reduce central fatigue would be to limit the entry of tryptophan into the brain by inhibiting the function of L-system transporter. Yamamoto and Newsholme (2000) used a specific L-system transporter inhibitor to attenuate the exercise induced increase in brain 5-HT and increase endurance exercise performance in rats. A similar experiment could be conducted with human subjects when a suitable inhibitor becomes available.

Appendix A. Statistical Analysis of Data from Chapter Two.

		Week	1	2	3	4	5	6
Group	T	No/D	4518(729)	4930(763)*	5598(641)*	5727(506)*	6084(663)*	6640(903)*
		Drug	306(69)	525(140)	777(242)*	792(219)*	832(283)*	712(192)*
	SC	No/S	2935(420)	4700(681)*	4301(508)*	6516(244)*	6645(403)*	7658(454)*
		Saline	3210(486)	4945(595)*	5672(464)*#	6911(342)*	7064(608)*	7377(463)*
	C	No/D	2172(218)&	1597(197)*	1823(227)	2206(220)	1470(160)*	1659(190)
		Drug	321(38)	242(59)	206(46)*	255(58)	230(67)	131(29)*

Table X. Endurance Performance for All Groups, Shown as Time to Fatigue. Endurance performance is shown as time to fatigue (s). Groups; T- training, SC- sham control and C- control. Data are group mean (+/- SEM). * indicates a significant difference ($p<0.05$) to week 1, # indicates a significant difference to endurance performance with m-CPP in the same week and & indicates a significant difference in undrugged (No/D) endurance performance between groups T and C in the same week.

Tests of Normality for All Data

Kolmogorov-Smirnov Test

Group	Treatment	Week	Kolmogorov-Smirnov Staistic	df	Sig.
Training	No Drug	1	0.266	3	<0.001
		2	0.353	3	<0.001
		3	0.234	3	<0.001
		4	0.216	3	<0.001
		5	0.177	3	<0.001
		6	0.179	3	<0.001
	Drug	1	0.244	3	<0.001
		2	0.207	3	<0.001
		3	0.231	3	<0.001
		4	0.323	3	<0.001
		5	0.309	3	<0.001
		6	0.370	3	<0.001
Sham Control	No Drug	1	0.342	3	<0.001
		2	0.233	3	<0.001
		3	0.236	3	<0.001
		4	0.274	3	<0.001
		5	0.265	3	<0.001
		6	0.236	3	<0.001

	Drug	1	0.243	3	<0.001
		2	0.175	3	<0.001
		3	0.263	3	<0.001
		4	0.184	3	<0.001
		5	0.385	3	<0.001
		6	0.285	3	<0.001
Control	No Drug	1	0.195	3	<0.001
		2	0.351	3	<0.001
		3	0.212	3	<0.001
		4	0.327	3	<0.001
		5	0.216	3	<0.001
		6	0.343	3	<0.001
	No Drug	1	0.280	3	<0.001
		2	0.369	3	<0.001
		3	0.328	3	<0.001
		4	0.352	3	<0.001
		5	0.181	3	<0.001
		6	0.289	3	<0.001

Comparison of Undrugged Endurance Performances in the Training Group

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 2 vs. Week 1	Negative Ranks	3	400.67	1400.00
	Positive Ranks	8	600.50	5200.00
	Ties	0		
	Total	11		
Week 3 vs. Week 1	Negative Ranks	3	400.67	1400.00
	Positive Ranks	8	600.50	5200.00
	Ties	0		
	Total	11		
Week 4 vs. Week 1	Negative Ranks	5	300.00	1500.00
	Positive Ranks	6	800.50	5100.00
	Ties	0		
	Total	11		
Week 5 vs. Week 1	Negative Ranks	2	400.00	800.00
	Positive Ranks	9	600.44	5800.00
	Ties	0		
	Total	11		

Week 6 vs. Week 1	Negative Ranks	3	300.00	900.00
	Positive Ranks	8	700.13	5700.00
	Ties	0		
	Total	11		

Wilcoxon Signed Rank Test Statistics

Comparison	Wk 2 – Wk 1	Wk 3 – Wk 1	Wk 4 – Wk 1	Wk 5 – Wk 1	Wk 6 – Wk 1
Z	-100.689	-100.689	-100.600	-200.223	-200.134
Sig. (2-tailed)	0.091	0.091	0.110	0.026	0.033

Comparison of Drugged Endurance Performances in the Training Group.

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 2 vs. Week 1	Negative Ranks	5	400.20	2100.00
	Positive Ranks	6	700.50	4500.00
	Ties	0		
	Total	11		
Week 3 vs. Week 1	Negative Ranks	2	100.50	300.00
	Positive Ranks	9	700.00	6300.00
	Ties	0		
	Total	11		
Week 4 vs. Week 1	Negative Ranks	0	00.00	00.00
	Positive Ranks	11	600.00	6600.00
	Ties	0		
	Total	11		
Week 5 vs. Week 1	Negative Ranks	3	300.67	1100.00
	Positive Ranks	8	600.88	5500.00
	Ties	0		
	Total	11		
Week 6 vs. Week 1	Negative Ranks	3	300.67	1100.00
	Positive Ranks	7	600.29	4400.00

	Ties	0
	Total	10

Wilcoxon Signed Rank Test Statistics

Comparison	Wk 2 – Wk 1	Wk 3 – Wk 1	Wk 4 – Wk 1	Wk 5 – Wk 1	Wk 6 – Wk 1
Z	-100.067	-200.667	-200.934	-100.956	-100.682
Sig. (2-tailed)	0.286	0.008	0.003	0.050	0.093

Comparison of Undrugged with Drugged Endurance Performances in the Training Group.

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 1	Negative Ranks	2	300.00	600.00
	Positive Ranks	4	300.75	1500.00
	Ties	0		
	Total	6		
Week 2	Negative Ranks	3	400.67	1400.00
	Positive Ranks	3	200.33	700.00
	Ties	0		
	Total	6		
Week 3	Negative Ranks	2	200.50	500.00
	Positive Ranks	4	400.00	1600.00
	Ties	0		
	Total	6		
Week 4	Negative Ranks	2	300.00	600.00
	Positive Ranks	3	300.00	900.00
	Ties	0		
	Total	5		
Week 5	Negative Ranks	0	00.00	00.00
	Positive Ranks	4	200.50	1000.00
	Ties	0		

	Total	4		
Week 6	Negative Ranks	3	200.33	700.00
	Positive Ranks	2	400.00	800.00
	Ties	0		
	Total	5		

Wilcoxon Signed Rank Test Statistics

Comparison	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Z	-0.943	-0.734	-100.153	-0.405	-100.826	-0.135
Sig. (2-tailed)	0.345	0.463	0.249	0.686	0.068	0.893

Comparison of Drugged Endurance Performances in the Control Group.

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 2 vs. Week 1	Negative Ranks	8	500.25	4200.00
	Positive Ranks	2	600.50	1300.00
	Ties	0		
	Total	10		
Week 3 vs. Week 1	Negative Ranks	9	500.67	5100.00
	Positive Ranks	1	400.00	400.00
	Ties	0		
	Total	10		
Week 4 vs. Week 1	Negative Ranks	7	500.71	4000.00
	Positive Ranks	3	500.00	1500.00
	Ties	0		
	Total	10		
Week 5 vs. Week 1	Negative Ranks	8	500.38	4300.00
	Positive Ranks	2	600.00	1200.00
	Ties	0		
	Total	10		

Week 6 vs. Week 1	Negative Ranks	10	500.50	5500.00
	Positive Ranks	0	00.00	00.00
	Ties	0		
	Total	10		

Wilcoxon Signed Rank Test Statistics

Comparison	Wk 2 – Wk 1	Wk 3 – Wk 1	Wk 4 – Wk 1	Wk 5 – Wk 1	Wk 6 – Wk 1
Z	-100.478	-200.395	-100.274	-100.580	-200.803
Sig. (2-tailed)	0.139	0.017	0.203	0.114	0.005

Comparison of Undrugged Endurance Performances in the Control Group.

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 2 vs. Week 1	Negative Ranks	9	500.89	5300.00
	Positive Ranks	1	200.00	200.00
	Ties	0		
	Total	10		
Week 3 vs. Week 1	Negative Ranks	8	500.75	4600.00
	Positive Ranks	2	400.50	900.00
	Ties	0		
	Total	10		
Week 4 vs. Week 1	Negative Ranks	5	400.80	2400.00
	Positive Ranks	4	500.25	2100.00
	Ties	0		
	Total	9		
Week 5 vs. Week 1	Negative Ranks	7	500.00	3500.00
	Positive Ranks	1	100.00	100.00
	Ties	0		
	Total	8		
Week 6 vs. Week 1	Negative Ranks	5	500.40	2700.00
	Positive Ranks	3	300.00	900.00

	Ties	0
	Total	8

Wilcoxon Signed Rank Test Statistics

Comparison	Wk 2 – Wk 1	Wk 3 – Wk 1	Wk 4 – Wk 1	Wk 5 – Wk 1	Wk 6 – Wk 1
Z	-200.599	-100.886	-00.178	-200.380	-100.260
Sig. (2-tailed)	0.009	0.059	0.859	0.017	0.208

Comparison of Drugged Endurance Performances Between the Training and Control Groups.

Wilcoxon Signed Rank Test

Comparison	Measure	N	Mean Rank	Sum of Ranks
Week 1	Negative Ranks	3	600.00	1800.00
	Positive Ranks	7	500.29	3700.00
	Ties	0		
	Total	10		
Week 2	Negative Ranks	6	600.17	3700.00
	Positive Ranks	4	400.50	1800.00
	Ties	0		
	Total	10		
Week 3	Negative Ranks	8	600.25	5000.00
	Positive Ranks	2	200.50	500.00
	Ties	0		
	Total	10		
Week 4	Negative Ranks	10	600.20	6200.00
	Positive Ranks	1	400.00	400.00
	Ties	0		
	Total	11		
Week 5	Negative Ranks	9	500.56	5000.00
	Positive Ranks	1	500.00	500.00
	Ties	0		
	Total	10		
Week 6	Negative Ranks	8	500.13	4100.00

	Positive Ranks	1	400.00	400.00
	Ties	0		
	Total	9		

Wilcoxon Signed Rank Test Statistics

Comparison	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Z	-00.968	-00.968	-200.293	-200.578	-200.293	-200.192
Sig. (2-tailed)	0.333	0.333	0.022	0.010	0.022	0.028

Correlation Between Undrugged and Drugged Endurance Performance for the Training Group.

Correlations

Week	Measure	Statistic
Week 2	Correlation Coefficient	0.592
	Sig. (1-tailed)	0.027
	N	11
Week 2	Correlation Coefficient	0.682
	Sig00. (1-tailed)	0.010
	N	11
Week 3	Correlation Coefficient	0.673
	Sig. (1-tailed)	0.012
	N	11
Week 4	Correlation Coefficient	0.364
	Sig. (1-tailed)	0.136
	N	11
Week 5	Correlation Coefficient	0.109
	Sig. (1-tailed)	0.375
	N	11
Week 6	Correlation Coefficient	0.612
	Sig. (1-tailed)	0.030
	N	10

Appendix B. Informed Consent Form for Chapter Three.

Informed Consent Form

Chief Investigators:	Dan Dwyer BHSc (Hons.) Dr. John Flynn
Faculty:	School of Health Science
Contact phone numbers:	D.Dwyer 07 5594 8372

Changes in the Serotonergic Activity in Response to Physical Training.

The Study.

<u>The purpose.</u>

A chemical in the brain called serotonin, has been shown to cause fatigue and to decrease exercise performance in animals and humans. Some animal studies have shown that an adaptation to serotonin occurs after exercise training, that allows improved exercise performance. The present project is the first training study examining this adaptation in humans.

There are also two studies that have shown differences in the way trained and untrained people cope with the fatiguing effects of serotonin during exercise. These studies lend support to the idea that humans tolerate serotonin better as a result of exercise training.

The mechanism of this adaptation is not understood. There are two likely mechanisms that explain this adaptation. We are investigating one of these mechanisms:

A decrease in serotonin receptor sensitivity occurs as a result of exercise training.

We are specifically interested in how the receptors for serotonin change as a result of exercise training. When serotonin attaches to one of its receptors in the brain it exerts its effects. The number and sensitivity of these receptors determines the severity of the effect, fatigue in this case. The changes in these receptors are interesting because they are semi-permanent and may effect other systems in the body. For example, serotonin also affects or appetite, body temperature and mood.

<u>Your involvement.</u>

In summary, there are three forms of assessment in this research. The first is a short exercise test, which involves you cycling on a stationary bicycle for about 12 minutes at a high intensity until you are fatigued. The second form of assessment is a longer exercise test, which requires you to cycle on a stationary bicycle for 1-2 hours at a low intensity until you are fatigued. Lastly you will be asked to take a drug (Buspirone) which stimulates serotonin receptors in the brain, and causes the release of a hormone called prolactin. This procedure will also require the

withdrawal of blood samples from you. This study also includes 10 weeks of exercise training on a stationary bicycle.

At this point we would like to point out that your involvement in this research is entirely voluntary. If you have decided now or at any point in the future that you do not want to participate then you need only tell us and you can stop. You are not obliged to participate or to continue your participation in this research.

Should you choose to participate in this research you will partake in the following procedures.

1. You will be given a questionnaire about your health and you will be asked to undertake an examination by a physician (Dr. John Flynn) to make sure that you are fit for exercise and that are not at risk of an adverse reaction to the drug we will administer to you (Buspirone). If you do not prove to be normal and healthy and provide no extra risk of adverse reaction to the drug, you will not be able to participate in this research. All information and test results will remain in the confidence of Dr. John Flynn.

2. You will be shown the sort of testing you will undergo and have a chance experience these tests if you like. This instruction session will take place in a laboratory at the university to simulate as closely as possible the real procedures.

3. You will then be given a fitness test to see how fit you are. This fitness test is a graded exercise test that requires you to cycle in a stationary bike progressively harder until volitional exhaustion. This is the only test in the study that requires you to exercise with maximal effort and lasts for about 12 minutes. This test will be used to determine your current exercise capacity and the intensity that you will cycle at during training

4. You will also be asked to perform an exercise test during which you will cycle on a stationary bicycle for 1-2 hours at a moderate intensity. This test is used to measure your endurance capacity.

5. Measurement of you serotonin receptor sensitivity will be performed in the following manner.

   You will be taken to Dr John Flynn's medical practice in the morning of the day of testing after fasting from midnight the night before. An indwelling catheter will be inserted into your forearm vein by a nurse or Dr. John Flynn. This a very small flexible tube that stays in you arm to allow blood samples to be taken. There is a prick sensation when the tube is inserted but there is no linger pain or discomfort even when subsequent blood samples are taken. Baseline blood samples will be taken, followed by a 60mg dose Buspirone, **administered orally** by the nurse or Dr. John Flynn. Samples of blood will be taken every 30 minutes from the indwelling catheter for 4 hours. Dr. John Flynn will be within 30sec. walk during the test and will be available to provide medical support should the need arise. This procedure will be repeated once again within 3-7 days.

   Each testing procedure will take about 4 hours to complete. You will be seated throughout the procedure and will be driven home, work or university after the test. Reading material will be provided to occupy you during this procedure. You are free to do anything during this time except anything

strenuous or stressful. Either the investigator or the nurse will be present the whole time to answer any questions you may have.

The blood samples that are taken will be labeled with a code and stored in the freezer for analysis at a later date. The samples will be analysed for their prolactin content, which indicates the sensitivity of the serotonin receptors in your brain.

6. The results of the first fitness test (3), we will prescribe the exercise program that you will be asked to follow for 10 weeks. This will involve cycling for 40 minutes 3 times per week at a target heart rate that would be described as vigorous. Periodically throughout the training period your target training intensity will be adjusted as your fitness improves.

7. After this training period you will be required to perform another assessment of your receptor sensitivity described in step 5.

8. You will also repeat the exercise tests described in steps 3 & 4.

In summary, your involvement in this study requires a commitment to

- A medical checkup
- 30 x ½ hour training sessions
- 4 x exercise tests and
- 4 x Buspirone challenge tests

You will be able to stop your participation in these procedures at any time throughout the study.

The Risks.

The Exercise Tests

There is a small risk associated with performing the fitness test, which involves maximal effort on your behalf. This is the same risk associated with any vigorous non-contact sport or exercise where you reach your maximum heart rate. This risk is minimised by the health screening process you will go through, and by the presence of trained staff monitoring your condition at all times and providing the necessary support in the event of a mishap. These trained staff know what symptoms indicate that you are not tolerating the exercise well.

The endurance test requires only sub-maximal effort and poses a similar but smaller risk.

Blood Sampling

Sampling blood from a forearm vein is a safe procedure performed routinely Dr. John Flynn's clinic. This procedure will be performed by a nurse or doctor who is certified to do so. You will experience a small prick when the needle is being inserted into your arm. This procedure occurs once during the initial medical check up and 4 times during the receptor assessment (a total of 5 pricks).

The Drug- Buspirone

Buspirone is a prescription drug found in any pharmacy. It is prescribed for people who have trouble controlling their anxiety. It has the effect of making you feel relaxed and calm. We will not be administering Buspirone to you because we think you have trouble controlling your anxiety. We are using Buspirone because it allows us to measure the sensitivity of the serotonin receptors in your brain.

If you take the drug the most likely symptoms that you will experience are sensations of calm and relaxation. However, with any drug there is a chance of an adverse reaction.

The most common adverse reactions to Buspirone and rates of appearance are

- dizziness, insomnia, nervousness, drowsiness and a light headed feeling (3.4%)
- gastrointestinal disturbances- primarily nausea (1.2%)
- and miscellaneous disturbances- primarily headache and fatigue (1.1%)

For comparison the rate of adverse reactions to Rhinocort Nasal Decongestant, a non prescription pharmaceutical available without prescription, are

- nasal irritation, itching of throat and larynx, sore throat, dry mucous membranes, dry mouth, sneezing after spraying, increased sputum, haemorrhagic secretion or nose bleeding, nasal crust, cough, dyspnoea, head ache dizziness and tiredness. (> 1%)
- bad taste, ear ache, loss of appetite, stomach disorder, nausea, skin itching, urticaria, tremor and sedation (1 - 0.2%)

The investigators will do everything possible to ensure you do not experience these symptoms. We will make sure that there is nothing in your medical history that will increase the risk of an adverse reaction. If you do have an adverse reaction to Buspirone, there will be a medical doctor available to attend to you and provide any support you will need until you are deemed fit to leave the clinic. You will not be required to continue with Buspirone trails if you have an adverse reaction. To remove any risk of an accident that may be caused by the effects of the drug, you should not drive immediately after administration, therefore you will be driven home, to work or university.

The Benefits.

For You

This study will provide you with a medical check up from a physician including ECG analysis (heart function). The results of the exercise tests will give you an indication of your improvement in exercise capacity. You will also receive a personalised, scientifically based exercise program with careful supervision and regular reassessment for 10 weeks. At the completion of the study you will be fitter than when you began and will enjoy the associated health benefits. You will also be offered advice on the continuation of your training program after the study has completed.

For Science

Exercise has also been investigated as an adjunct therapy for depression. Depression is thought to be related to an imbalance in serotonin regulation in the brain. The current trend in the pharmacological treatment of depression is to use a promising class of drugs that alter serotonin metabolism in the brain (Selective Serotonin Reuptake Inhibitors). Preliminary animal studies indicate that exercise may offer similar benefits. This study will further the understanding of how serotonin activity changes in humans with exercise training and how exercise may benefit suffers of depression.

Chronic Fatigue Syndrome (CFS) is also thought to be a disorder of the serotonergic system. At least one study has shown up regulation of receptors in subjects diagnosed with CFS. Preliminary results from animal studies suggest that exercise training causes a down regulation of serotonin receptors. If this response also occurs in human subjects, then exercise may be a useful adjunct therapy for suffers of CFS.

Confidentiality.

All records of test results will be kept securely in a locked filing cabinet in the chief investigator office. No one other than the chief investigators and you will see this information. The results will be analysed by coding each individual with number that will not identify you. Any information presented to anyone else will be coded so that it does not identify you.

Your voluntary consent.

It is important that if you give your consent it is given freely. You are under no obligation what so ever to agree to participate in this research.

It is also important that if you agree to participate in this research that you understand what will be required of you and the tests that are involved.

If you have any questions about these procedures feel free to ask any of the investigators at any time now or throughout the research. You can ask us directly or you can contact us on the telephone numbers on the first page of this document.

If you wish to discontinue as a subject then you should feel free to do so at any time. The will be no penalty or consequences if you decide to withdraw- this is your right at any time.

Feedback.

You will receive regular information and feedback about all of your test results and your training progress. There will be many opportunities to ask questions at all stages throughout this research.

After the study is completed, our scientific findings will be presented to the group in an informative lecture. We will also offer you the chance to see and discuss your personal test results.

I agree to participate in this study and acknowledge that the procedures, including the risks and benefits, have been explained to me and I have had the opportunity to ask questions.

Signatures.

…….
Chief Investigator(s) date

…….
Participant date

……..
Witness date

Appendix C. Adverse Reactions to Buspirone

The physical risks associated with the use of Buspirone are minimal. Buspirone is a schedule 4 drug and has been approved for sale and general prescription by the Health Department in Australia.

The risk of adverse reactions will be reduced by screening the subjects before they take the drug in the manner described previously in this application. The following information on types and rates of adverse reactions is taken from studies that used prolonged doses of Buspirone. It is expected that as the present study uses only two single doses, the risk of adverse reaction is low.

The most common adverse reactions to Buspirone and rates of appearance are

- dizziness, insomnia, nervousness, drowsiness and a light headed feeling (3.4%)
- gastrointesinal disturbances- primarily nausea (1.2%) and
- miscellaneous disturbances- primarily headache and fatigue (1.1%)

For comparison the rate of adverse reactions to Rhinocort Nasal Decongestant are

- nasal irritation, itching of throat and larynx, sore throat, dry mucous membranes, dry mouth, sneezing after spraying, increased sputum, haemorrhagic secretion or nose bleeding, nasal crust, cough, dyspnoea, head ache dizziness and tiredness. (> 1%)
- bad taste, ear ache, loss of appetite, stomach disorder, nausea, skin itching, urticaria, tremor and sedation (1 - 0.2%)

“Buspirone has not shown potential for a drug abuse and dependence based upon limited human and animal studies” (Carroll, 1999).

Appendix D. Statistical Analysis of Data from Chapter Three

Tests of Normality of Personal and Performance Data

Kolmogorov-Smirnov Test

Group	Measure	Sample	Kolmogorov-Smirnov statistic	df	Sig.
Control	Body Weight	Pre	0.287	6	0.134
		Post	0.344	6	0.025
	VO_{2max}	Pre	0.188	6	0.200
		Post	0.226	6	0.200
	Endurance Performance	Pre	0.257	6	0.200
		Post	0.262	6	0.200
Training	Body Weight	Pre	0.236	6	0.200
		Post	0.258	6	0.200
	VO_{2max}	Pre	0.168	6	0.200
		Post	0.183	6	0.200
	Endurance Performance	Pre	0.215	6	0.200
		Post	0.351	6	0.020

Comparison of Change in Body Mass and Performance.

Paired Samples T-Test

Measure	Group	Mean Difference	Mean Std. Deviation	Mean Std. Error	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Body Mass (kg)	Training	0.86	3.029	0.8744	-1.0579	2.7912	0.991	11	0.343
	Control	-1.97	3.077	1.1633	-4.8178	.8750	-1.695	6	0.141
VO_{2max}	Training	-0.466	0.176	0.0508	-.5784	-.3547	-9.179	11	**0.000**
	Control	0.06	0.177	0.067	-9.7549E-02	.2310	0.994	6	.359
Endurance Performance	Training	-81.0	39.5	11.923	-107.7481	-54.6155	-6.809	10	**0.000**
	Control	-14.0	36.0	13.942	-48.9739	19.2596	-1.066	6	0.328

Comparison of Time Course Changes in Prolactin Response to Buspirone for the Training and Control groups.

Tests of Normality

Kolmogorov-Smirnov Test

Group	Pre or Post	Sample Time (min)	Kolmogorov-Smirnov statistic	df	Sig.
Control	Pre	0	0.228	7	0.200
		30	0.452	7	0.000
		60	0.329	7	0.022
		90	0.311	7	0.040
		120	0.477	7	0.000
		150	0.427	7	0.000
		180	0.348	7	0.010
		210	0.186	7	0.200
		240	0.208	7	0.200
	Post	0	0.142	7	0.200
		30	0.401	7	0.001
		60	0.295	7	0.066
		90	0.284	7	0.091
		120	0.382	7	0.003
		150	0.178	7	0.200
		180	0.197	7	0.200
		210	0.162	7	0.200
		240	0.293	7	0.071
Training	Pre	0	0.378	12	0.000
		30	0.208	12	0.162
		60	0.100	12	0.200
		90	0.192	12	0.200
		120	0.157	12	0.200
		150	0.204	12	0.180
		180	0.258	12	0.027
		210	0.331	12	0.001
		240	0.399	12	0.000
	Post	0	0.305	12	0.003
		30	0.227	12	0.088
		60	0.151	12	0.200
		90	0.155	12	0.200
		120	0.235	12	0.067
		150	0.207	12	0.166
		180	0.286	12	0.008
		210	0.328	12	0.001
		240	0.348	12	0.000

Repeated Measures ANOVA

Group	Factor	df	F	Sig.
Control	Sample Time	8	2.822	0.012
	(No)Training	1	0.391	0.55
	Sample Time x (No)Training	8	0.921	0.508
Training	Sample Time	8	18.642	< 0.001
	Training	1	0.69	0.798
	Sample Time x Training	8	1.014	0.432

Comparison of the Area Under the Curve (AUC) for the Prolactin Response to Buspirone for the Training and Control Groups.

Tests of Normality

Kolmogorov-Smirnov Test

Group	Pre-Post	Kolmogorov-Smirnov Statistic	df	Sig.
Control	Pre	0.347	7	0.011
	Post	0.305	7	0.048
Training	Pre	0.196	7	0.200
	Post	0.163	7	0.200

Wilcoxon Signed Ranks Test for Control Group Data

Ranks

		N	Mean Rank	Sum of Ranks
Pre vs. Post	Negative Ranks	2	4.00	8.00
	Positive Ranks	3	2.33	7.00
	Ties	2		
	Total	7		

Wilcoxon Signed Ranks Test Statistics

	Pre vs. Post
Z	-0.135
Sig. (2-tailed)	0.893

Paired Samples T-Test for Training Group Data

Group	Mean Difference	Std. Deviation	Mean Std. Error	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
				Lower	Upper			
Training	162.31	2141.92	618.32	-1198.60	1523.22	0.26	11	0.798

Appendix E. Informed Consent Form for Chapter Four

Informed Consent Form

The Effect of an Increase in Serotonergic Activity on Neuromuscular Function

Chief Investigators: Dan Dwyer BHSc (Hons.)
Gary Dennis

Faculty: School of Health Science

Contact phone numbers: D.Dwyer 07 5594 8372

Details of procedures

We wish to add one simple test procedure to the existing test protocol. This procedure involves performing a series of biceps curls (elbow flexion) to determine the maximum force you can generate and the endurance capacity of your arm.

You will be asked to perform duplicate testing sessions to assess the fatiguing effect of the drug (Buspirone). A testing session will involve the subject performing biceps curls (elbow flexion) while seated on a piece of gym equipment designed for this type of exercise.

- The first two tests examine your reaction time and hand eye coordination. They are computer based tests and are very simple to perform.
- The functional tests involve having two electrodes placed on the skin over your right biceps muscle for the purpose of measuring EMG (muscle activity). A third electrode is placed on your elbow. This is a non-invasive, painless procedure that is used in our laboratories on a daily basis. The electrodes measure muscle activity only and do not deliver any charge to the subject.
- You will be asked to perform a series of 3 maximal biceps curls to determine the maximum voluntary contractile (MVC) force you can generate. The peak force will be measured by using a force transducer connected to the bar and EMG will be recorded by a computer
- You will rest for ~3 minutes and then perform 3 sustained (isometric) biceps curl at ~50% of your MVC force until the point of fatigue.
- Finally you will be required to perform a sustained biceps contraction lasting for 30s

Throughout this exercise test, standard safety procedures set out by the American College of Sports Medicine and those sanctioned by Griffith University will be adhered to. These procedures include supervision by trained staff.

Risk:

There is a minimal risk associated with performing exhaustive biceps curls. You may experience some residual muscle soreness, but no more than one might expect from vigorous exercise.

Standard requirements for confidentiality and feedback of results will be adhered to.

Signature of participant ……. Date:

Signature of witness ……. Date:

Signature of investigator ……. Date:

Appendix F. Statistical Analysis of Data from Chapter Four

Analysis of Functional Data

Tests of Normality for Data

Kolmogorov-Smirnov Test

	Measure	Statistic	df	Sig.
Placebo	Reaction Time (pre)	.208	10	.200
	Hand Eye Coordination (pre)	.153	10	.200
	Maximal Voluntary Isometric Contractile Force (pre)	.193	10	.200
	Isometric Neuromuscular Control (pre)	.164	10	.200
	Isometric Muscular Endurance Capacity (pre)	.245	10	.092
	Reaction Time (post)	.132	10	.200
	Hand Eye Coordination (post)	.277	10	.029
	Maximal Voluntary Isometric Contractile Force (post)	.213	10	.200
	Isometric Neuromuscular Control (post)	.175	10	.200
	Isometric Muscular Endurance Capacity (post)	.212	10	.200
Drug	Reaction Time (pre)	.206	10	.200
	Hand Eye Coordination (pre)	.251	10	.075
	Maximal Voluntary Isometric Contractile Force (pre)	.164	10	.200
	Isometric Neuromuscular Control (pre)	.187	10	.200
	Isometric Muscular Endurance Capacity (pre)	.166	10	.200
	Reaction Time (post)	.159	10	.200
	Hand Eye Coordination (post)	.240	10	.107
	Maximal Voluntary Isometric Contractile Force (post)	.203	10	.200
	Isometric Neuromuscular Control (post)	.181	10	.200
	Isometric Muscular Endurance Capacity (post)	.159	10	.200

Tests of Normality for Effect Size Data

Kolmogorov-Smirnov Test

Placebo	Reaction Time	.226	10	.157
	Hand Eye Coordination	.135	10	.200
	Maximal Voluntary Isometric Contractile Force	.270	10	.038
	Isometric Neuromuscular Control	.148	10	.200
	Isometric Muscular Endurance Capacity	.132	10	.200
Drug	Reaction Time	.177	10	.200
	Hand Eye Coordination	.150	10	.200
	Maximal Voluntary Isometric Contractile Force	.246	10	.088
	Isometric Neuromuscular Control	.137	10	.200
	Isometric Muscular Endurance Capacity	.151	10	.200

Descriptive Table of "Pre" Treatment Functional Data

Measure	Mean	Std. Deviation	SEM
Reaction Time- pre placebo	291.2	18.67738	5.90631
Reaction Time- pre drug	293.1	30.19363	9.54807
Hand Eye Coordination- pre placebo	798.4	86.69256	27.41459
Hand Eye Coordination- pre drug	787.6	91.21793	28.84564
Maximal Voluntary Isometric Contractile Force- pre placebo	276.5	40.30784	12.74646
Maximal Voluntary Isometric Contractile Force- pre drug	289.6	48.67854	15.39351
Isometric Neuromuscular Control- pre placebo	.6903	.25907	.08193
Isometric Neuromuscular Control- pre drug	.6564	.23110	.07308
Isometric Muscular Endurance Capacity- pre placebo	5243.0	1358.51062	429.5987
Isometric Muscular Endurance Capacity- pre drug	5572.1	1681.20647	531.6441

T-tests of "Pre" Treatment Functional Data

Measure (Pre placebo vs. Pre drug)	t	df	Sig. (2-tailed)
Reaction Time	-.271	9	.792
Hand Eye Coordination	.575	9	.579
Maximal Voluntary Isometric Contractile Force	-2.127	9	.062
Isometric Neuromuscular Control	.345	9	.738
Isometric Muscular Endurance Capacity (post)	-1.808	9	.104

Descriptive Table of Effect Size of Functional Data

Measure	Mean	Std. Deviation	SEM
Reaction Time- placebo	14.1000	25.11949	7.94348
Reaction Time- drug	18.2000	13.66911	4.32255
Hand Eye Coordination- placebo	-7.4000	50.39885	15.93752
Hand Eye Coordination- drug	23.5000	50.53327	15.98002
Maximal Voluntary Isometric Contractile Force- placebo	-8.7000	9.23821	2.92138
Maximal Voluntary Isometric Contractile Force- drug	-22.3000	12.52597	3.96106
Isometric Neuromuscular Control- placebo	-.1231	.18181	.05749
Isometric Neuromuscular Control- drug	.1339	.26217	.08291
Isometric Muscular Endurance Capacity- placebo	-26.7000	395.42861	125.0455
Isometric Muscular Endurance Capacity- drug	-407.2000	431.82141	136.5539

T-tests of Effect Size of Functional Data

Measure (Post placebo – Pre placebo vs. Post drug – Pre drug)	t	df	Sig. (2-tailed)
Reaction	-.535	9	.605
Hand Eye Coordination	-1.510	9	.165
Maximal Voluntary Isometric Contractile Force	2.896	9	.018
Isometric Neuromuscular Control	-2.419	9	.039
Reaction Time	3.834	9	.004

Analysis of EMG Data from the MVIC-F and INC Tests

Tests of Normality for MVIC-F and INC EMG Data

Kolmogorov-Smirnov Test

Measure	Statistic	df	Sig.
Amplitude pre placebo	.222	10	.178
Amplitude pre drug	.148	10	.200
Amplitude post placebo	.164	10	.200
Amplitude post drug	.163	10	.200
Median Pre placebo	.257	10	.061
Median pre drug	.158	10	.200
Median post placebo	.364	10	.000
Median Post drug	.156	10	.200
Bandwidth pre placebo	.268	10	.041
Bandwidth pre drug	.168	10	.200
Bandwidth post placebo	.158	10	.200
Bandwidth post drug	.167	10	.200

Descriptive Table of "Pre" Treatment EMG Data from the MVIC-F Test

Measure	Mean	Std. Deviation	SEM
Amplitude Pre placebo	1.4870	.7426	.2348
Amplitude Pre drug	1.7610	.6556	.2073
Median Pre placebo	72.0680	12.1958	3.8566
Median Pre drug	64.6670	6.6673	2.1084
Bandwidth Pre placebo	61.3990	23.2227	7.3437
Bandwidth Pre drug	50.4660	10.8632	3.4352

T-tests of Pre of EMG Data from MVIC-F tests

Measure (Pre placebo vs. Pre drug)	t	df	Sig. (2-tailed)
Amplitude	-1.113	9	.294
Median Frequency	1.652	9	.133
Frequency Bandwidth	1.592	9	.146

Descriptive Table of Effect Size EMG Data from the MVIC-F Test

Measure	Mean	Std. Deviation	SEM
Amplitude placebo	-.2990	.4878	.1542
Amplitude drug	-0.016	.4428	.1400
Median placebo	.2660	4.7579	1.5046
Median drug	.7660	7.4673	2.3614
Bandwidth placebo	6.8670	9.1290	2.8868
Bandwidth drug	.6180	20.0043	6.3259

T-tests of Effect Size of EMG Data from MVIC-F Tests

Measure (placebo vs. drug)	t	df	Sig. (2-tailed)
Amplitude	-1.970	9	.080
Median Frequency	-.135	9	.895
Frequency Bandwidth	.793	9	.448

Isometric Neuromuscular Control EMG Data Analysis

Tests of Normality for Isometric Neuromuscular Control Data

Kolmogorov-Smirnov Test

Measure	Statistic	df	Sig.
Median pre placebo	.170	10	.200
Median pre drug	.190	10	.200
Median post placebo	.143	10	.200
Median post drug	.190	10	.200
Bandwidth pre placebo	.252	10	.072
Bandwidth pre drug	.196	10	.200
Bandwidth post placebo	.172	10	.200
Bandwidth post drug	.204	10	.200

Descriptive Table of Isometric Neuromuscular Control "Pre" EMG Data

Measure	Mean	Std. Deviation	SEM
Median pre placebo	51.5000	14.1152	4.4636
Median pre drug	52.7670	16.4904	5.2147
bandwidth pre placebo	100.5660	34.9210	11.0430
bandwidth pre drug	99.8670	37.1581	11.7504

T-tests of "Pre" Means of EMG Data from Isometric Neuromuscular Control Tests

Measure	t	df	Sig. (2-tailed)
Median Frequency	-.225	9	.827
Frequency Bandwidth	.049	9	.962

Tests of Normality for Isometric Neuromuscular Control Effect Size Data

Kolmogorov-Smirnov Test

Measure	Statistic	df	Sig.
Median drug	.389	10	.000
Bandwidth drug	.308	10	.008
Median placebo	.235	10	.124
Bandwidth pre placebo	.194	10	.200

Descriptive Table of Isometric Neuromuscular Control Effect Size EMG Data

Measure	Mean	Std. Deviation	SEM
Median drug	-8.2330	16.1679	5.1127
Bandwidth drug	-4.4010	17.9909	5.6892
Median placebo	-15.7660	36.7600	11.6245
Bandwidth pre placebo	-9.6670	46.3903	14.6699

T-tests of Effect Size of EMG Data from Isometric Neuromuscular Control Tests

Measure	t	df	Sig. (2-tailed)
Median Frequency	-.462	9	.655
Frequency Bandwidth	-.319	9	.757

Isometric Muscular Endurance Capacity EMG Data Analysis

Tests of Normality for Isometric Muscular Endurance Capacity Data

Kolmogorov-Smirnov Test

Time Sample Interval	Measure	Statistic	df	Sig.
3-6 s	Amplitude pre placebo	0.200	10	0.200
	Amplitude pre drug	0.133	10	0.200
	Amplitude post placebo	0.227	10	0.154
	Amplitude post drug	0.161	10	0.200
	Median Pre placebo	0.252	10	0.072
	Median pre drug	0.205	10	0.200
	Median post placebo	0.192	10	0.200
	Median Post drug	0.175	10	0.200
	Bandwidth pre placebo	0.179	10	0.200
	Bandwidth pre drug	0.211	10	0.200
	Bandwidth post placebo	0.172	10	0.200
	Bandwidth post drug	0.159	10	0.200
13-16 s	Amplitude pre placebo	0.254	10	0.066
	Amplitude pre drug	0.180	10	0.200
	Amplitude post placebo	0.220	10	0.188
	Amplitude post drug	0.238	10	0.114
	Median Pre placebo	0.275	10	**0.031**
	Median pre drug	0.121	10	0.200
	Median post placebo	0.160	10	0.200
	Median Post drug	0.133	10	0.200
	Bandwidth pre placebo	0.225	10	0.165
	Bandwidth pre drug	0.192	10	0.200
	Bandwidth post placebo	0.169	10	0.200
	Bandwidth post drug	0.182	10	0.200
23-26 s	Amplitude pre placebo	0.206	10	0.200
	Amplitude pre drug	0.213	10	0.200
	Amplitude post placebo	0.186	10	0.200
	Amplitude post drug	0.204	10	0.200
	Median Pre placebo	0.195	10	0.200
	Median pre drug	0.200	10	0.200
	Median post placebo	0.209	10	0.200

	Median Post drug	0.139	10	0.200
	Bandwidth pre placebo	0.135	10	0.200
	Bandwidth pre drug	0.245	10	0.092
	Bandwidth post placebo	0.223	10	0.171
	Bandwidth post drug	0.214	10	0.200

Repeated Measures ANOVA of Isometric Muscular Endurance Capacity EMG Data

Time Sample Interval	Measure	Factor	F	Sig.
3-6 s	Amplitude	Time	0.380	0.55
		Treatment	0.664	0.43
		Time x Treatment	0.205	0.66
	Median	Time	0.741	0.41
		Treatment	30.894	0.08
		Time x Treatment	50.215	**0.04**
	Bandwidth	Time	0.217	0.65
		Treatment	40.326	0.06
		Time x Treatment	30.056	0.11
13-16 s	Amplitude	Time	30.285	0.10
		Treatment	0.041	0.84
		Time x Treatment	10.823	0.21
	Median	Time	0.001	0.97
		Treatment	20.848	0.12
		Time x Treatment	10.277	0.28
	Bandwidth	Time	0.276	0.61
		Treatment	40.167	0.07
		Time x Treatment	0.002	0.96
23-26 s	Amplitude	Time	0.235	0.63
		Treatment	0.003	0.95
		Time x Treatment	0.989	0.34
	Median	Time	10.022	0.33
		Treatment	10.412	0.26
		Time x Treatment	20.891	0.12

	Bandwidth	Time	0.701	0.42
		Treatment	30.742	0.08
		Time x Treatment	0.001	0.97

References

Acworth, I. Nicholass, J., Morgan, B., and Newsholme, E. (1986) Effect of sustained exercise on concentrations of plasma aromatic and branched-chain amino acids and brain amines. Biochem Biophys Res Commun. 137(1):149-53.

Aghajanian, G.K and Lakoski, JM. (1984) Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K+ conductance. Brain Res. 305(1): 181-5.

Aghajanian, G.K. and Vandermaelen, C.P. (1986) Specific systems of the reticular core: serotonin. In *Handbook of Physiology, The Nervous System. Intrinsic regulatory systems of the brain.* Bethesda, MD. Am Physiol Soc. 4(1): 237-56.

Ahlenius, S., Kaur, P. and Salmi, P. (1997) Biphasic effects of 8-OH-DPAT on endurance of treadmill performance in the male rat. Eur Neuropsychopharmacol. 7(2):89-94.

Akai, T., Takahashi, M., Nakada, Y., Ohnishi, R., Ikoma, Y. and Yamaguchi, M. (1991) Anxiolytic effects of lisuride and its agonistic action to central 5-HT1A receptors. Nippon Yakurigaku Zasshi. 97(4): 209-20.

Akil, H. and Liebeskind, J.C. (1975) Monoaminergic mechanisms of stimulation-produced analgesia. Brain Res. 94(2):279-96.

Franklin, B. Ed. (2001) *American College of Sports Medicine Guidelines for Exercise Testing and Prescription*, 6th Edition. Lippincott, Williams and Wilkins, Philadelphia.

Asmussen, E. (1979) Muscle fatigue. Med Sci Sports Exerc. 11:313-321.

Audet, M., Descarries, L. and Doucet, G. (1989) Quantified regional and laminar distribution of the serotonin innervation in the anterior half of the adult cerebral cortex. J Chem Neuroanat. 2:29-44.

Avela, J. and Komi, P.V. (1998) Reduced stretch reflex sensitivity and muscle stiffness after long-lasting stretch-shortening cycle exercise in humans. Eur J Appl Physiol Occup Physiol. 78(5):403-10.

Avela, J., Kyrolainen, H. and Komi, P.V. (2001) Neuromuscular changes after long-lasting mechanically and electrically elicited fatigue. Eur J Appl Physiol. 85(3-4):317-25.

Bagdy, G., Szemeredi, K., Kanyicska, B. and Murphy, D. (1989) Different serotonin receptors mediate blood pressure, heart rate, plasma catecholamine and prolactin responses to m-chlorophenylpiperazine in conscious rats. Journal of Pharmacology and Experimental Therapeutics. 250(1): 72-78.

Bailey, S. P. Davis, J. M., and Ahlborn, E. N. (1992) Effect of increased brain serotonergic activity on endurance performance in the rat. Acta Physiol Scand. 145(1):75-6.

Bailey, S. P. Davis, J. M., and Ahlborn, E. N. (1993a) Neuroendocrine and substrate responses to altered brain 5-HT activity during prolonged exercise to fatigue. J Appl Physiol. 74(6):3006-12.

Bailey, S. P. Davis, J. M., and Ahlborn, E. N. (1993b) Serotonergic agonists and antagonists affect endurance performance in the rat. Int J Sports Med. 14(6):330-3.

Bakheit, A.M., Behan, P.O., Dinan, T.G., Gray, C.E., and O'Keane, V. (1992) Possible upregulation of hypothalamic 5-hydroxytryptamine receptors in patients with postviral fatigue syndrome. BMJ. 304(6833)1010-2.

Balaban, E.P. (1992) Sports anemia. Clin Sports Med. 11(2):313-25.

Baldwin, K.M. (2000) Research in the exercise sciences: where do we go from here? J Appl Physiol. 88(1):332-6.

Barbeau, H. and Rossignol, S. (1991) Initiation and modulation of the locomotor pattern in the adult chronic spinal cat by noradrenergic, serotonergic and dopaminergic drugs. Brain Res. 546(2):250-60.

Barnes, N.M. and Sharp, T. (1999) A review of central 5-HT receptors and their function. Neuropharmacology. 38(8):1083-152.

Bassett, D.R Jr and Howley, E.T. (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc. 32(1):70-84.

Beato, M. and Nistri, A. (1998) Serotonin-induced inhibition of locomotor rhythm of the rat isolated spinal cord is mediated by the 5-HT1 receptor class. Proc R Soc Lond B Biol Sci. 265(1410):2073-80.

Bhagat, B. and Wheeler, N. (1973) Effect of amphetamine on the swimming endurance of rats. Neuropharmacology. 12(7):711-3.

Bigland-Ritchie, B., Johansson, R., Lippold, O.C. and Woods, J.J. (1983) Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. J Neurophysiol. 50(1): 313-24.

Bigland-Ritchie, B.R., Cafarelli, E. and Vollestad, N.K. (1986a) Fatigue of submaximal static contractions. Acta Physiol Scand Suppl. 556: 137-48.

Bigland-Ritchie, B.R., Dawson, N.J., Johansson, R.S. and Lippold O.C. (1986b) Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. J Physiol (Lond). 379: 451-459.

Blomstrand, E., Celsing, F., and Newsholme, E. A. (1988) Changes in plasma concentrations of aromatic and branched-chain amino acids during sustained exercise in man and their possible role in fatigue. Acta Physiol Scand. 133(1):115-21.

Blomstrand, E., Perrett, D., Parry Billings, M. and Newsholme, E. A. (1989) Effect of sustained exercise on plasma amino acid concentrations and on 5-hydroxytryptamine metabolism in six different brain regions in the rat. Acta Physiol Scand. 136(3):473-81.

Bobker, D.H. and Williams, J.T. (1990) Ion conductances affected by 5-HT receptor subtypes in mammalian neurons.Trends Neurosci. 13(5):169-73.

Brasil-Neto, J.P., Pascual-Leone, A., Valls-Sole, J., Cammarota, A., Cohen, L.G. and Hallett, M. (1993) Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. Brain Res. 93(1):181-4.

Bridge, M.W., Marvin, G., Thompson, C.E., Sharma, A., Jones, D.A. and Kendall, M.J. (2001) Quantifying the 5-HT1A agonist action of buspirone in man. Psychopharmacology. 158(3): 224-9.

Broocks, A., Meyer, T., George, A., Hillmer-Vogel, U., Meyer, D., Bandelow, B., Hajak, G., Bartmann, U., Gleiter, C.H. and Ruther, E. (1999) Decreased neuroendocrine responses to meta-chlorophenylpiperazine (m-CPP) but normal responses to ipsapirone in marathon runners. Neuropsychopharmacology. 20(2):150-61.

Brown, B.S., Payne, T., Kim, C., Moore, G., Krebs, P. and Martin, W. (1979) Chronic response of rat brain norepinephrine and serotonin levels to endurance training. J Appl Physiol. 46(1):19-23.

Calders, P., Matthys, D., Derave, W. and Pannier, J.L. (1999) Effect of branched-chain amino acids (BCAA), glucose, and glucose plus BCAA on endurance performance in rats. Med Sci Sports Exerc. 31(4):583-7.

Carroll, P. Ed. (1999) *MIMS Annual.* Intercontinental Medical Statistics (Australasia). Crows Nest, Australia.

Casini, V. (1998) Worker deaths by electrocution. National Institute for Occupational Safety and Health. Pub. No. 98-131.

Cazalets, J.R., Sqalli-Houssaini, Y. and Clarac, F. (1992) Activation of the central pattern generators for locomotion by serotonin and excitatory amino acids in neonatal rat. J Physiol. 455:187-204.

Chaouloff, F. (1989) Physical exercise and brain monoamines: a review. Acta Physiol Scand. 137(1):1-13.

Chaouloff, F. (1994) Influence of physical exercise on 5-HT1A receptor and anxiety-related behaviours. Neurosci Lett. 176(2):226-30.

Chaouloff, F. (1997) Effects of acute physical exercise on central serotonergic systems. Med Sci Sports Exerc. 29(1):58-62.

Chaouloff, F., Elghozi, J.L., Guezennec, Y. and Laude, D. (1985) Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-hydroxytryptamine metabolism of the rat. Br J Pharmacol. 86(1):33-41.

Chaouloff, F., Kennett, G.A., Serrurrier, B., Merino, D. and Curzon, G. (1986a) Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. J Neurochem. 46(5): 1647-50.

Chaouloff, F., Laude, D., Guezennec, Y., and Elghozi, J. L. (1986b) Motor activity increases tryptophan, 5-hydroxyindoleacetic acid, and homovanillic acid in ventricular cerebrospinal fluid of the conscious rat. J Neurochem. 46(4):1313-6.

Chaouloff, F., Laude, D., Merino, D., Serrurrier, B., Guezennec, Y., and Elghozi, J. L. (1987) Amphetamine and alpha-methyl-p-tyrosine affect the exercise-induced imbalance between the availability of tryptophan and synthesis of serotonin in the brain of the rat. Neuropharmacology. 26(8):1099-106.

Coccaro, E.F., Gabriel, S. and Siever, L.J. (1990) Buspirone challenge: preliminary evidence for a role for central 5-HT1a receptor function in impulsive aggressive behavior in humans. Psychopharmacol Bull. 26(3): 393-405.

Coccaro, E.F., Gabriel, S., Mahon, T., Macaluso, J. and Siever, L.J. (1992) Response to: Effect of buspirone on prolactin secretion is not mediated by 5-HT1a receptor stimulation. Arch Gen Psychiatry. 49:163-164.

Cowen, P.J., Anderson, I.M. and Grahame-Smith, D.G. (1990) Neuroendocrine effects of azapirones. J Clin Psychopharmacol. 10(3 Suppl):21S-25S.

Cunliffe, A., Obeid, O.A. and Powell-Tuck, J. (1998) A placebo controlled investigation of the effects of tryptophan or placebo on subjective and objective measures of fatigue. Eur J Clin Nutr. 52(6):425-30.

Curzon, G., Friedel, J. and Knott, P.J. (1973) The effect of fatty acids on the binding of tryptophan to plasma protein. Nature. 242(5394): 198-200.

Davies, C.A., Mann, D.M., Sumpter, P.Q. and Yates, P.O. (1987) A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J Neurol Sci. 78(2): 151-64.

Davis, J. M. and Bailey, S. P. (1997) Possible mechanisms of central nervous system fatigue during exercise. Med Sci Sports Exerc. 29(1):45-57.

Davis, J. M., Bailey, S. P., Woods, J. A., Galiano, F. J., Hamilton, M. T. and Bartoli, W. P. (1992) Effects of carbohydrate feedings on plasma free tryptophan and branched-chain amino acids during prolonged cycling. Eur J Appl Physiol. 65(6):513-9.

Dey, S. (1994) Physical exercise as a novel antidepressant agent: possible role of serotonin receptor subtypes. Physiol Behav. 55(2):323-9.

Dey, S., Singh, R.H. and Dey, P.K. (1992) Exercise training: significance of regional alterations in serotonin metabolism of rat brain in relation to antidepressant effect of exercise. Physiol Behav. 52(6):1095-9.

De Luca, C.J. (1984) Myoelectrical manifestations of localized muscular fatigue in humans. Crit Rev Biomed Eng. 11(4):251-79.

Dimitrova N. and Dimitrov G. (2003) Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. J Electromyogr Kinesiol. 13(1):13-36.

Di Renzo, G., Amoroso, S., Taglialatela, M., Canzoniero, L., Basile, V., Fatatis, A. and Annunziato, L. (1989) Pharmacological characterization of serotonin receptors involved in the control of prolactin secretion. Eur J Pharmacol. 162(2):371-3.

Eison, A.S. and Temple, D.L. Jr. (1986) Buspirone: review of its pharmacology and current perspectives on its mechanism of action. Am J Med. 80(3B): 1-9.

Elam, M., Svensson, T. H., and Thoren, P. (1987) Brain monoamine metabolism is altered in rats following spontaneous, long-distance running. Acta Physiol Scand. 130(2):313-6.

El-Yassir, N., Fleetwood-Walker, S.M. and Mitchell, R. (1988) Heterogeneous effects of serotonin in the dorsal horn of rat: the involvement of 5-HT1 receptor subtypes. Brain Res. 456(1): 147-58.

Enoka, R.M. (1995) Mechanisms of muscle fatigue: Central factors and task dependency. Journal of Electromyography and Kinesiology. 5(3):141-149.

Farris, J.W., Hinchcliff, K.W., McKeever, K.H., Lamb, D.R. and Thompson, D.L. (1998) Effect of tryptophan and of glucose on exercise capacity of horses. J Appl Physiol. 85(3):807-16.

Fernstrom M. and Fernstrom J. (1993) Large changes in serum free tryptophan levels do not alter brain tryptophan levels: studies in streptozotocin-diabetic rats. Life Sci. 52(11):907-16.

Fernstrom, J.D. and Wurtman, R.J. (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. Science. 173(992):149-52.

Fischer, H.G., Hollmann, W., De Meirleir, K., Chaouloff, F., Elghozi, J. L., Guezennec, Y., Laude, D., and Dey, S. (1991) Exercise changes in plasma tryptophan fractions and relationship with prolactin. Effects of conditioned running on plasma, liver and brain tryptophan and on brain 5-hydroxytryptamine metabolism of the rat. Physical exercise as a novel antidepressant agent: possible role of serotonin receptor subtypes. Int J Sports Med. 12(5):487-9.

Fornal, C.A., Metzler, C.W., Marrosu, F., Ribiero-do-Valle, L.E. and Jacobs, B.L. (1996) A subgroup of dorsal raphe serotonergic neurons in the cat is strongly activated during oral-buccal movements. Brain Res. 716(1-2):123-33.

Gandevia, S. C. (1988) Physiological accompaniments of human muscle fatigue. Aust Paediatr J. 24 Suppl 1104-8.

Gandevia, S.C., Allen, G. M. and McKenzie, D.K. (1995) Central fatigue. Critical issues, quantification and practical implications. Adv Exp Med Biol. 384:281-94.

Gerin, C., Legrand, A., Privat, A., Heyes, M. P., Garnett, E. S., Coates, G., Chaouloff, F., Kennett, G. A., Serrurrier, B., Merino, D. and Curzon, G. (1994) Study of 5-HT release with a chronically implanted microdialysis probe in the ventral horn of the spinal cord of unrestrained rats during exercise on a treadmill. J Neurosci Methods. 52(2)129-41.

Gill, N.D., Shield, A., Blazevich, A.J., Zhou, S. and Weatherby, R.P. (2000) Muscular and cardiorespiratory effects of pseudoephedrine in human athletes. Br J Clin Pharmacol. 50(3):205-13.

Gleason, S.D. and Shannon, H.E. (1998) Meta-chlorophenylpiperazine induced changes in locomotor activity are mediated by 5-HT1 as well as 5-HT2C receptors in mice. Eur J Pharmacol. 341(2-3):135-8.

Gollnick, P.D. (1982) Peripheral factors as limitations to exercise performance. Can J App Spt Sci. 7(1):14-21.

Gomez-Merino, D., Bequet, F., Berthelot, M., Riverain, S., Chennaoui, M. and Guezennec, C.Y. (2001) Evidence that the branched-chain amino acid L-valine prevents exercise-induced release of 5-HT in rat hippocampus. Int J Sports Med. 22(5):317-22.

Grillner, S. and Wallen, P. (1985) Central pattern generators for locomotion, with special reference to vertebrates. Annu Rev Neurosci. 8:233-61.

Hamon, M., Bourgoin, S., Artaud, F. and El Mestikawy, S. (1981) The respective roles of tryptophan uptake and tryptophan hydroxylase in the regulation of serotonin synthesis in the central nervous system. J Physiol (Paris). 77(2-3):269-79.

Heyes, M.P., Garnett, E.S. and Coates G. (1988) Nigrostriatal dopaminergic activity is increased during exhaustive exercise stress in rats. Life. 42(16):1537-42.

Hill, A.V. and Lupton, H. (1923) Muscular exercise, lactic acid and the supply and utilisation of oxygen. Q.J.Med. 16:135-171.

Hjorth, S. and Carlsson, A. (1982) Buspirone: effects on central monoaminergic transmission--possible relevance to animal experimental and clinical findings. Eur J Pharmacol. 83(3-4):299-303.

Hoffmann, P., Elam, M., Thoren, P., Hjorth, S., Gerin, C., Becquet, D. and Privat, A. (1994) Effects of long-lasting voluntary running on the cerebral levels of dopamine, serotonin and their metabolites in the spontaneously hypertensive rat. Life Sci. 54(13):855-61.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R. and Humphrey, P.P. (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev. 46(2):157-203.

Inoue, K., Yamazaki, H., Manabe, Y., Fukuda, C. and Fushiki, T. (1998) Release of a substance that suppresses spontaneous motor activity in the brain by physical exercise. Physiol Behav. 64(2):185-90.

Jacobs, B.L. (1991) Serotonin and behavior: emphasis on motor control. J Clin Psychiatry. 52 Suppl:17-23.

Jacobs, B.L. and Azmitia, E.C. (1992) Structure and function of the brain serotonin system. Physiol Rev. 72(1):165-229.

Jacobs, B.L. and Fornal, C.A. (1993) 5-HT and motor control: a hypothesis. Trends Neurosci. 16(9):346-52.

Jacobs, B.L. and Fornal, C.A. (1997) Serotonin and motor activity. Curr Opin Neurobiol. 7(6):820-5.

Jacobs, B.L and Fornal, C.A. (1999) Activity of serotonergic neurons in behaving animals. Neuropsychopharmacology. 21(2 Suppl):9S-15S.

Jakeman, P. M., Hawthorne, J. E., Maxwell, S. R., Kendall, M. J., Holder, G., Chance, W. T., Balasubramaniam, A., Thomas, I. and Fischer, J. E. (1994) Evidence for downregulation of hypothalamic 5-hydroxytryptamine receptor function in endurance-trained athletes. Exp Physiol. 79(3):461-4.

Jones, M. and Tunstall Pedoe, D.S. (1989) Blood doping- a literature review. Br J Sports Med. 23(2):84-8.

Kent-Braun, J.A. (1999) Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. Eur J Appl Physiol Occup Physiol. 80(1):57-63.

Kiehn, O. and Kjaerulff, O. (1996) Spatiotemporal characteristics of 5-HT and dopamine-induced rhythmic hindlimb activity in the in vitro neonatal rat. J Neurophysiol. 75(4):1472-82.

Kotz, Y.M., Rodionov, I. M. and Sitnikov, V. I. (1978) On the mechanism of an Increase in Muscle Performance and of Vasodilation during Emotional Stress in Man. Pflugers Arch. 373:211-218.

Krogh-Lund, C. and Jorgensen, K. (1993) Myo-electric fatigue manifestations revisited: power spectrum, conduction velocity, and amplitude of human elbow flexor muscles during isolated and repetitive endurance contractions at 30% maximal voluntary contraction. Eur J Appl Physiol Occup Physiol. 66(2):161-73.

Lamb, D.R. (1984) *Physiology of exercise: responses & adaptations*. 2nd Ed. Macmillan, New York.

Latash, M.L. (1998) *Neurophysiological basis of movement*. Human Kinetics, Champaign.

Lehmann, M., Huonker, M., Dimeo, F., Heinz, N., Gastmann, U., Treis, N., Steinacker, J. M., Keul, J., Kajewski, R. and Haussinger, D. (1995) Serum amino acid concentrations in nine athletes before and after the 1993 Colmar ultra triathlon. Int J Sports Med. 16(3):155-9.

Leone, M., Attanasio, A., Croci, D., Ferraris, A., D'Amico, D., Grazzi, L., Nespolo, A. and Bussone, G. (1998) 5-HT1A receptor hypersensitivity in migraine is suggested by the m-chlorophenylpiperazine test. Neuroreport. 9(11):2605-8.

Lepers R., Maffiuletti N., Rochette L., Brugniaux J., Millet GY. (2002) Neuromuscular fatigue during a long-duration cycling exercise. J Appl Physiol. 92(4):1487-93.

Lloyd, A.R., Gandevia, S.C. and Hales, J.P. (1991) Muscle performance, voluntary activation, twitch properties and perceived effort in normal subjects and patients with the chronic fatigue syndrome. Brain. 114(Pt 1A):85-98.

MacKay-Lyons M. (2002) Central pattern generation of locomotion: a review of the evidence. Phys Ther. 82(1): 69-83.

Maclaren, D.P, Gibson, H., Parry-Billings, M. and Edwards, R.H. (1989) A review of metabolic and physiological factors in fatigue. Exerc Sport Sci Rev. 17:29-66.

MacLean, D.A. and Graham, T.E. (1993) Branched-chain amino acid supplementation augments plasma ammonia responses during exercise in humans. J Appl Physiol. 74(6):2711-7.

Marieb, E. (2001). *Human Anatomy and Physiology*. Benjamin/Cummings. Redwood City.

Marvin, G., Sharma, A., Aston, W., Field, C., Kendall, M. J. and Jones, D.A. (1997) The effects of buspirone on perceived exertion and time to fatigue in man. Exp Physiol. 82(6):1057-60.

Maskall, D.D., Zis, A.P., Lam, R.W., Clark, C.M. and Kuan, A.J. (1995) Prolactin response to buspirone challenge in the presence of dopaminergic blockade. Biol Psychiatry. 38(4): 235-9.

McAllister-Williams, R.H. and Massey, A.E. (2003) EEG effects of buspirone and pindolol: a method of examining 5-HT(1A) receptor function in humans. Psychopharmacology. 166(3): 284-93.

McMenamy, H. (1965) Binding of indole analogues to human serum albumin. Effects of fatty acids. J Biol Chem. 240(11):4235-43.

Meeusen, R., Piacentini, M.F., Van Den Eynde, S., Magnus, L. and De Meirleir, K. (2001) Exercise performance is not influenced by a 5-HT reuptake inhibitor. Int J Sports Med. 22(5):329-36.

Meeusen, R., Roeykens, J., Magnus, L., Keizer, H., DeMeirleir, K., Szetchman, H., Talangbayan, H., Canaran, G., Dai, H. and Eilam, D. (1997) Endurance performance in humans: The effect of a dopamine precursor or a specific serotonin (5-HT2A/2C) antagonist. International Journal of Sports Medicine. 18(8):571-577.

Meeusen, R., Thorre, K., Chaouloff, F., Sarre, S., De Meirleir, K., Ebinger, G. and Michotte, Y. (1996) Effects of tryptophan and/or acute running on extracellular 5-HT and 5-HIAA levels in the hippocampus of food-deprived rats. Brain Res. 740(1-2):245-52.

Meltzer, H.Y. (1992) Effect of buspirone on prolactin secretion is not mediated by 5-HT1a receptor stimulation. Arch Gen Psychiatry. 49: 163.

Meltzer, H.Y., Flemming, R. and Robertson, A. (1983) The effect of buspirone on prolactin and growth hormone secretion in man. Arch Gen Psychiatry. 40(10):1099-102.

Meltzer, H.Y. and Maes, M. (1994) Effects of buspirone on plasma prolactin and cortisol levels in major depressed and normal subjects. Biol Psychiatry. 35(5):316-23.

Mitchell, J.B., Paquet, A.J., Pizza, F.X., Starling, R.D., Holtz, R.W. and Grandjean, P.W. (1996) The effect of moderate aerobic training on lymphocyte proliferation. Int J Sports Med. 17(5):384-9.

Miyachi, M. and Tabata, I. (1992) Relationship between arterial oxygen desaturation and ventilation during maximal exercise. J Appl Physiol. 73(6):2588-91.

Monti, J.M., Jantos, H., Monti, D. and Alvarino, F. (2000) Dorsal raphe nucleus administration of 5-HT1A receptor agonist and antagonists: effect on rapid eye movement sleep in the rat. Sleep Res Online. 3(1): 29-34.

Moskowitz, H. and Smiley, A. Effects of chronically administered buspirone and diazepam on driving-related skills performance. J Clin Psychiatry. 43(12 Pt 2):45-55.

Naoi, M., Maruyama, W., Takahashi, T., Ota, M. and Parvez, H. (1994) Inhibition of tryptophan hydroxylase by dopamine and the precursor amino acids. Biochem Pharmacol. 48(1): 207-11.

Nash, J.F., Meltzer, H.Y. and Gudelsky, G.A. (1988) Antagonism of serotonin receptor mediated neuroendocrine and temperature responses by atypical neuroleptics in the rat. Eur J Pharmacol. 151(3):463-9.

Neuhauser, H., Laakmann, G. and Knossalla, A. (1988) Neuroendocrine effects of Buspiroine. 16th CNIP Congress Psychopharmacology. 96[suppl]: 39-41.

Newsholme, E. and Leech, A. (1983) *Biochemistry for the Medical Sciences.* John Wiley & Sons. Chichester.

Nybo L., Nielsen B., Blomstrand E., Moller K., Secher N. (2003) Neurohumoral responses during prolonged exercise in humans. J Appl Physiol. 95(3):1125-31.

Oberlander, C., Demassey, Y., Verdu, A., Van de Velde, D., and Bardelay, C. (1987) Tolerance to the serotonin 5-HT1 agonist RU 24969 and effects on dopaminergic behaviour. Eur J Pharmacol. 139(2):205-14.

O'Hanlon, J.F. (1991) Review of buspirone's effects on human performance and related variables. Eur Neuropsychopharmacol. 1(4):489-501.

Pannier, J.L., Bouckaert, J.J. and Lefebvre, R.A. (1995) The antiserotonin agent pizotifen does not increase endurance performance in humans. Eur J Appl Physiol. 72(1-2):175-8.

Pardridge, W.M. (1977) Kinetics of competitive inhibition of neutral amino acid transport across the blood-brain barrier. J Neurochem. 28(1):103-8.

Parkhouse, W.S., McKenzie, D.C. (1984) Possible contribution of skeletal muscle buffers to enhanced anaerobic performance: a brief review. Med Sci Sports Exerc. 16(4):328-38.

Paul, G.L., Rokusek, J.T., Dykstra, G.L., Boileau, R.A. and Layman, D.K. (1996) Preexercise meal composition alters plasma large neutral amino acid responses during exercise and recovery. American Journal of Clinical Nutrition. 64(5):778-786.

Pieron, H. Le Probleme physiologique du sommeil. Masson Ed., Paris. 1912. In Romanowski, W. and Grabiec, S. (1974) The role of serotonin in the mechanism of central fatigue. Acta Physiol Pol. 25(2):127-34.

Porter, R.J., McAllister-Williams, R.H., Jones, S. and Young, A.H. (1999) Effects of dexamethasone on neuroendocrine and psychological responses to L-tryptophan infusion. Psychopharmacology (Berl). 143(1):64-71.

Rang, H.P., Dale, M.M. and Ritter J.M. (1999) *Pharmacology* 4th Ed. Churchill Livingstone, New York.

Rapport, M.M. (1948) Serum vasoconstrictor (serotonin) V. The presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. J Biol Chem. 180:961-969.

Rapport, M.M. and Green, A.A. (1948) Serum vasoconstrictor (serotonin) IV. Isolation and characterization. J Biol Chem. 176:1243-1251.

Reith, M.E. (1990) 5-HT3 receptor antagonists attenuate cocaine-induced locomotion in mice. Eur J Pharmacol. 186(2-3):327-30.

Ribeiro-do-Valle, L.E., Metzler, C.W. and Jacobs, B.L. (1991) Facilitation of masseter EMG and masseteric (jaw-closure) reflex by serotonin in behaving cats. Brain Res. 550(2):197-204.

Roberts, H. and Wright, M. (1981) The effects of chronic section of dorsal roots on the responsiveness of motoneurones to 5-hydroxytryptamine and a substance P analogue. Br J Pharmacol. 73(3):589-94.

Roed, A. (1988) Fatigue during continuous 20 Hz stimulation of the rat phrenic nerve diaphragm preparation. Acta Physiol Scand. 134(2):217-21.

Romanowski, W. and Grabiec S. (1974) The Role of Serotonin in the Mechanism of Central Fatigue. Acta Physiol Pol. 25(2):127-134.

Romanowski, W. and Janota-Lukaszewska, J. (1967) Studies on an inhibitory substance in the extracts of brains of rats subjected to acute physical effort. Acta Physiol Pol. 18(2):215-23.

Rudnick, G. and Clark, J. (1993) From synapse to vesicle: the reuptake and storage of biogenic amine neurotransmitters. Biochim Biophys Acta. 1144(3):249-63.

Saltin, B., Gollnick, P.D., Eriksson, B.O. and Piehel, K. (1972) Onset of Exercise Symposium, Toulouse. 63-76.

Samii, A., Wassermann, E.M., Ikoma, K., Mercuri, B. and Hallett, M. (1996) Characterization of postexercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation. Neurology. 46(5):1376-82.

Schaffer, K. and Klausnitzer, W. (1988) Single dose study on buspirone versus diazepam in volunteers. Arzneim Forsch. 38:282-287. In O'Hanlon, J.F. (1991) Review of buspirone's effects on human performance and related variables. Eur Neuropsychopharmacol. 1(4):489-501.

Schwenk, E. (1965) Hoppe Seyler’s Zschr F Angew Cheme. In Romanowski, W. and Grabiec, S. (1974) The role of serotonin in the mechanism of central fatigue. Acta Physiol Pol. 25(2):127-34.

Secher, N.H. (1987) Motor unit recruitment: A pharmacological approach. Med Sports Sci. 26: 152-162.

Segal, M. (1980) The action of serotonin in the rat hippocampal slice preparation. J Physiol Lond. 303: 443-456.

Seguin, L., Liscia, P., Guezennec, Y. and Fillion, G. (1998) Effects of moderate and intensive training on functional activity of central 5-HT1B receptors in the rat substantia nigra. Acta Physiol Scand. 162(1):63-8.

Segura, R. and Ventura, J.L. (1988) Effect of L-tryptophan supplementation on exercise performance. Int J Sports Med. 9(5):301-5.

Seppala, T., Ranta, T. and Shrotriya, R.C. Serum prolactin levels after buspirone in man. Med Biol. 65(1):61-3.

Sharpe, M., Clements, A., Hawton, K., Young, A.H., Sargent, P. and Cowen, P.J. (1996) Increased prolactin response to buspirone in chronic fatigue syndrome. Journal of Affective Disorders. 41(1):71-76.

Sills, M.A., Lucki, I., Frazer, A. (1985) Development of selective tolerance to the serotonin behavioral syndrome and suppression of locomotor activity after repeated administration of either 5-MeODMT or mCPP. Life Sci. 36(26):2463-9.

Simonsen, E. (Ed). (1971) *Physiology of Work Capacity and Fatigue*. C. C. Thomas Publisher Springfield.

Sjodin, B. and Svedenhag, J. (1985) Applied physiology of marathon running. Sports Med. 2(2):83-99.

Soares, J., Naffah Mazzacoratti, M.G., Cavalheiro, E.A., Chaouloff, F., Laude, D. and Elghozi, J.L. (1994) Increased serotonin levels in physically trained men. Physical exercise: evidence for differential consequences of tryptophan on 5-HT synthesis and metabolism in central serotonergic cell bodies and terminals. Braz J Med Biol Res. 27(7):1635-8.

Spampinato, U., Esposito, E. and Samanin, R. (1985) Serotonin agonists reduce dopamine synthesis in the striatum only when the impulse flow of nigro-striatal neurons is intact. J Neurochem. 45(3): 980-2.

Speciale, S.G., Miller, J.D., McMillen, B.A. and German, D.C. (1986) Activation of specific central dopamine pathways: locomotion and footshock. Brain Res Bull. 16(1): 33-8.

Spina, R.J. (1999) Cardiovascular adaptations to endurance exercise training in older men and women. Exerc Sport Sci Rev. 27:317-32.

Spriet, L.L., Soderlund, K., Bergstrom, M. and Hultman, E. (1987) Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. J Appl Physiol. 62(2):616-21.

Stensrud, T., Ingjer, F., Holm, H. and Stromme, S.B. (1992) L-tryptophan supplementation does not improve running performance. Int J Sports Med. 13(6):481-5.

Stephens J. and Taylor, A. (1973) Fatigue of maintained voluntary muscle contraction in man. J Physiol (Lond.). 220:1-18.

Sternbach, H. (1991) The serotonin syndrome. Am J Psychiatry. 148: 705-713.

Strachan, A.T. and Maughan, R.J. (1998) Platelet serotonin transporter density and related parameters in endurance-trained and sedentary male subjects. Acta Physiol Scand. 163(2): 165-71.

Struder, H.K., Hollmann, W., Platen, P., Duperly, J., Fischer, H. G. and Weber, K. (1996) Alterations in plasma free tryptophan and large neutral amino acids do not affect perceived exertion and prolactin during 90 min of treadmill exercise. Int J Sports Med. 17(2):73-9.

Struder, H.K., Hollmann, W., Platen, P., Wostmann, R., Weicker, H. and Molderings, G.J. (1999) Effect of acute and chronic exercise on plasma amino acids and prolactin concentrations and on [3H]ketanserin binding to serotonin2A receptors on human platelets. Eur J Appl Physiol Occup Physiol. 79(4):318-24.

Struder, H.K. and Weicker, H. (2001) Physiology and pathophysiology of the serotonergic system and its implications on mental and physical performance. Part I. Int J Sports Med. 22(7):467-81.

Tamasy, V., Koranyi, L. and Phelps, C.P. (1981) The role of dopaminergic and serotonergic mechanisms in the development of swimming ability of young rats. Dev Neurosci. 4(5):389-400.

Taylor, J.L. and Gandevia, S.C. (2001) Transcranial magnetic stimulation and human muscle fatigue. Muscle Nerve 24(1):18-29.

Thompson, P.A., Maes, M. and Meltzer, H.Y. (1994) Effect of the placebo control condition in neuroendocrine challenge studies. Psychiatry Res 52(3):317-26.

Ulrichsen, J., Partilla, J.S., Dax, E.M. (1992) Long-term administration of m-chlorophenylpiperazine (mCPP) to rats induces changes in serotonin receptor binding, dopamine levels and locomotor activity without altering prolactin and corticosterone secretion. Psychopharmacology (Berl). 107(2-3):229-35.

Vandermaelen, C.P. and Wilderman, R.C. (1986) Iontophoretic and systemic administration of the non-benzodiazepine anxiolytic drug buspirone causes inhibition of serotonergic dorsal raphe neurons in rats. Eur J Pharm. 129:23-130.

Van de Kar, L.D., Javed, A., Zhang, Y., Serres, F., Raap, D.K. and Gray, T.S. (2001) 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. J Neurosci. 21(10):3572-9.

Van de Kar, L.D., Lorens, S.A., Urban, J.H., Bethea, C.L. (1989) Effect of selective serotonin (5-HT) agonists and 5-HT2 antagonist on prolactin secretion. Neuropharmacology. 28(3):299-305.

Van Hall G., Raaymakers J., Saris W., Wagenmakers A. (1995) Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. J Physiol. 1;486(Pt 3):789-94.

Varnier, M., Sarto, P., Martines, D., Lora, L., Carmignoto, F., Leese, G.P. and Naccarato, R. (1994) Effect of infusing branched-chain amino acid during incremental exercise with reduced muscle glycogen content. Eur J Appl Physiol Occup Physiol. 69(1):26-31.

Veasey, S.C., Fornal, C.A., Metzler, C.W. and Jacobs, B.L. (1995) Response of serotonergic caudal raphe neurons in relation to specific motor activities in freely moving cats. J Neurosci. 15(7 Pt 2):5346-59.

Verger, P., Aymard, P., Cynobert, L., Anton, G. and Luigi, R. (1994) Effects of administration of branched-chain amino acids vs. glucose during acute exercise in the rat. Physiol-Behav. 55(3):523-6.

Vollestad, N.K. (1997) Measurement of human muscle fatigue. Journal of Neuroscience Methods. 74(2):219-227.

Wallis, D. (1994) 5-HT receptors involved in initiation or modulation of motor patterns: opportunities for drug development. Trends Pharmacol Sci. 15(8):288-92.

Wikstrom, M., Hill, R., Hellgren, J. and Grillner, S. (1995) The action of 5-HT on calcium-dependent potassium channels and on the spinal locomotor network in lamprey is mediated by 5-HT1A-like receptors. Brain Res. 678(1-2):191-9.

Wilson, W.M. and Marsden, C.A. (1996) In vivo measurement of extracellular serotonin in the ventral hippocampus during treadmill running. Behav Pharmacol. 7(1):101-104.

Wilson, W.M. and Maughan, R.J. (1992) Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. Exp Physiol. 77(6):921-4.

Wite, S., Fung, S., Jackson., D. and Imel, K. (1996) Serotonin, norepinephrine and associated nuropeptides: effects on somatic motoneurone excitability. Prog Brain Res. 107:183-199.

Woolley, D.W. and Shaw, E. (1954) A biochemical and pharmacological suggestion about certain mental disorders. Science. 119: 587-588.

Wurtman, R.J. (1983) Food consumption, neurotransmitter synthesis, and human behaviour. Experientia Suppl. 44:356-69.

Yamamoto, T. and Newsholme, E.A. (2000) Diminished central fatigue by inhibition of the L-system transporter for the uptake of tryptophan. Brain Res Bull. 52(1):35-8.

Young, S.N., Pihl, R.O. and Ervin, F.R. (1986) The effect of altered tryptophan levels on mood and behaviour in normal human males. Clin Neuropharmacol. 9 Suppl 4:516-8.

Yue, G. and Cole, K.J. (1992) Strength increases from the motor program: comparison of training with maximal voluntary and imagined muscle contractions. J Neurophysiol. 67(5):1114-23.