Central Fatigue
The Serotonin Hypothesis and Beyond

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Abstract

The original central fatigue hypothesis suggested that an exercise-induced increase in extracellular serotonin concentrations in several brain regions contributed to the development of fatigue during prolonged exercise. Serotonin has been linked to fatigue because of its well known effects on sleep, lethargy and drowsiness and loss of motivation. Several nutritional and pharmacological studies have attempted to manipulate central serotonergic activity during exercise, but this work has yet to provide robust evidence for a significant role of serotonin in the fatigue process. However, it is important to note that brain function is not determined by a single neurotransmitter system and the interaction between brain serotonin and dopamine during prolonged exercise has also been explored as having a regulative role in the development of fatigue. This revised central fatigue hypothesis suggests that an increase in central ratio of serotonin to dopamine is

associated with feelings of tiredness and lethargy, accelerating the onset of fatigue, whereas a low ratio favours improved performance through the maintenance of motivation and arousal. Convincing evidence for a role of dopamine in the development of fatigue comes from work investigating the physiological responses to amphetamine use, but other strategies to manipulate central catecholamines have yet to influence exercise capacity during exercise in temperate conditions. Recent findings have, however, provided support for a significant role of dopamine and noradrenaline (norepinephrine) in performance during exercise in the heat. As serotonergic and catecholaminergic projections innervate areas of the hypothalamus, the thermoregulatory centre, a change in the activity of these neurons may be expected to contribute to the control of body temperature whilst at rest and during exercise. Fatigue during prolonged exercise clearly is influenced by a complex interaction between peripheral and central factors.

The limits of performance during prolonged exercise have been the subject of numerous physiological and psychological studies. Fatigue has traditionally been attributed to the occurrence of a 'metabolic endpoint', where muscle glycogen concentrations are depleted, plasma glucose concentrations are reduced and plasma free fatty acid levels are elevated. However, the causes of fatigue are believed to be of both peripheral and central origin, therefore, fatigue should be acknowledged as a complex phenomenon influenced by both peripheral and central factors.[1,2]

The notion that the CNS is involved in the development of fatigue is not new. Early work by Alessandro Mosso (1904) crudely demonstrated a reduced capacity to perform repeated muscle contractions following a mental effort, resulting in the development of the term 'mental fatigue'.[3] Later, Romanowski and Grabiec[4] mentioned the possibility of centrally mediated fatigue during exercise. They linked serotonin to a possible inhibition of brain oxidoreductive processes, while others[5,6] highlighted the role of dopamine in the development of fatigue. Since these original works, many advances have been made to clarify the role of the CNS in the development of fatigue. While there have been a number of neurobiological mechanisms proposed to explain the apparent loss of neural drive referred to as central fatigue, the neurotransmitter hypothesis first put forward by Acworth et al.,[7] then later developed by Newsholme et al.,[8] has received the greatest academic recognition to date.

Many factors influence the capacity to perform prolonged exercise and the relative importance of these different factors varies depending on the duration of exercise, the intensity of the work, the mode of exercise and the environmental conditions. Therefore, when reconsidering the central fatigue hypothesis, it is important to reflect on all these elements. The purpose of this article is to review the possible cerebral responses during prolonged exercise and their possible connection to fatigue. We will evaluate the scientific evidence for the original central fatigue hypothesis by looking at the underlying neurobiological mechanism. This article will highlight other possible central factors that may be important in the development of fatigue, in particular when exercise is performed in a warm environment. As body temperature appears to be an important factor in the fatigue process under conditions of heat stress, the underlying neurochemical mechanisms implicated in the control of thermoregulation whilst at rest and during exercise will also be explored.

1. Fatigue: the Central Fatigue Hypothesis

Fatigue during prolonged exercise has been defined as the inability to maintain the required or expected power output that leads to a loss of performance in a given task.[9] Several lines of evidence indicate that fatigue develops gradually and there is a reduction in the maximal force a muscle can

produce from the outset of prolonged exercise. Therefore, it may be more useful to define fatigue as any exercise-induced reduction in the ability to exert muscle force or power regardless of whether or not the task can be sustained.[10] Since much of the work in this area is interested in the development of fatigue during prolonged exercise, fatigue under these conditions is often defined as a failure to continue working at a given exercise intensity.[11]

Factors thought to be important in the development of peripheral fatigue during prolonged exercise include the depletion of muscle glycogen,[12] which is thought to limit the rate of adenosine diphosphate rephosphorylation, and the progressive loss of body fluids resulting in increased cardiovascular, metabolic and thermoregulatory strain. The latter is particularly important when exercise is performed in warm ambient conditions, as it elevates body heat storage, accelerating the development of hyperthermia.[13] Thus, peripheral fatigue encompasses events that occur independently of the CNS, including disturbances to neuromuscular transmission, sarcolemma excitability and excitation-contraction coupling.

The central fatigue hypothesis is based on the assumption that during prolonged exercise the synthesis and metabolism of central monoamines, in particular serotonin, dopamine and noradrenaline (norepinephrine) are influenced. It was first suggested by Newsholme et al.[8] that during prolonged exercise increased brain serotonergic activity may augment lethargy and loss of drive, resulting in a reduction in motor unit recruitment. This, in turn, may influence the physical and mental efficiency of the exercising individual, factors that could be regarded as central fatigue.

The serotonergic system has been suggested as an important modulator of mood, emotion, sleep and appetite, and thus has been implicated in the control of numerous behavioural and physiological functions.[14] Serotonin is unable to cross the blood-brain barrier (BBB), therefore, cerebral neurons are required to synthesise it for themselves. The initial step in this process is the uptake of the amino acid tryptophan (TRP), across the BBB. TRP is the precursor for the synthesis of serotonin, and increased TRP availability to the serotonergic neurons results in an increase in cerebral serotonin levels, because the enzyme that converts TRP to serotonin (tryptophan hydroxylase) is not saturated under normal physiological conditions. Consequently, the transport of TRP into the brain is considered to be the rate-limiting step in the synthesis of serotonin, with an increase or decrease in brain TRP availability producing a corresponding change in the rate of serotonin synthesis within the CNS.[15]

It was initially believed that the concentration of plasma TRP was the only determinant of serotonin synthesis;[16] however, subsequent work has gradually revealed that the situation is complicated by additional factors relating to the transport of TRP across the BBB. TRP binds to albumin in the blood, a protein transporter shared with free-fatty acids (FFA), and in the systemic circulation only a small fraction (10–20%) is present as free TRP (f-TRP) at rest. There is limited evidence for a relationship between plasma f-TRP and brain TRP content under resting conditions.[17,18] However, during exercise, the concentration of plasma f-TRP, rather than total-TRP, has been identified as a key factor in determining the rate of cerebral TRP uptake during exercise, with a strong positive relationship reported between changes in plasma f-TRP and brain TRP content.[19] The apparent difference in the size of the TRP pool available for transport into the CNS may result from changes in cerebral blood flow. Cerebral blood flow is markedly increased to a large part of the brain during exercise[20] and this increase in blood velocity may limit the effectiveness of the large neutral amino acid carrier at removing TRP from albumin.

The mobilisation of FFA from adipose tissue by adrenaline (epinephrine)-stimulated lipolysis has been proposed to be important to the development of serotonin-mediated fatigue during prolonged exercise.[8] Plasma FFA concentrations typically increase progressively throughout prolonged low- to moderate-intensity exercise, particularly following a period of food deprivation (e.g. an overnight fast). Although there appears to be a near linear relationship between plasma FFA and the rate of FFA

utilisation,[21] this increase occurs as the mobilisation of FFA from the adipose tissue often slightly exceeds uptake by the working muscles.[22] When muscle and liver glycogen stores are nearing depletion, FFA mobilisation can increase disproportionately over the rate of transport into the muscle, resulting in a marked elevation in plasma FFA concentrations.[23] As FFA molecules bind to albumin, conformational changes occur that result in the liberation of TRP from its binding site,[24] consequently increasing the proportion of TRP circulating in a free form.

The entry of TRP into the brain competes with the transport of branched-chain amino acids (BCAA – leucine, isoleucine and valine) across the BBB, as they are mediated by the same carrier system.[25] This led to the suggestion that the plasma concentration ratio of TRP to competing amino acids is important in determining the rate of cerebral TRP uptake, with an increase in this ratio leading to increased brain TRP and serotonin content.[15] Because, prolonged exercise results in FFA release from adipose tissue, plasma concentrations of both FFA and f-TRP increase, producing a corresponding increase in cerebral serotonin levels. According to the hypothesis of Newsholme et al.,[8] this change in brain neurochemistry results in subjective sensations of lethargy and tiredness, causing an altered sensation of effort, perhaps a differing tolerance of pain/discomfort and a loss of drive and motivation to continue exercise.

Based on the literature presented above, the underlying mechanism behind the central fatigue hypothesis as proposed by Newsholme et al.[8] can be divided into two interrelated sections:

1. Under resting conditions, the majority of TRP circulates in the blood loosely bound to albumin, a transporter shared with f-FFA. The shift in substrate mobilisation occurring as exercise progresses causes an increase in plasma FFA concentration. This displaces TRP from binding sites on albumin, leading to a marked increase in f-TRP. f-TRP is then readily available for transport across the BBB.

2. Plasma BCAA concentrations either fall[26,27] or are unchanged[28] during prolonged exercise. Since f-TRP and BCAA share a common transporter across the BBB, a reduction in competing large-neutral amino acids would increase the uptake of TRP into the CNS.

It would be naive to believe that the only regulator of serotonin release and synthesis is the delivery of TRP to a serotonergic neuron. A number of subtle control factors have been proposed to influence serotonin synthesis, including the availability of oxygen and pteridine – cofactors that are required in the hydroxylation of TRP.[14] serotonin release is thought to be influenced by the activity of other neurotransmitter systems, including dopamine and GABA as well as cerebral glucose availability.[29] Additionally, increases in extracellular serotonin concentrations have been demonstrated to activate serotonin 5-HT$_{1A}$ (and potentially 5-HT$_{1B/1D}$) autoreceptors producing a negative feedback, normalising serotonin levels due to a reduction in the firing rate of the neuron.[30] An overview of the major serotonergic receptors studied in relation to central fatigue is presented in table I.

Furthermore, it is possible that the interaction between brain serotonin and dopamine during prolonged exercise could play a regulative role in the onset of fatigue.[31] Unknowingly, dopamine was one of the first neurotransmitters to be linked to central fatigue, through the study of amphetamine use. Animal studies[5,32,33] showed that increased brain dopamine activity occurs during prolonged physical activity and this may be important to exercise performance.[31] The association between exercise performance and dopaminergic activity becomes clear when we consider that dopamine plays an important role in motivation, memory, reward and attention. Evidence suggests that animals are motivated to perform behaviours that stimulate dopamine release in the ventral tegmental area[34] and addiction is a common feature of a number of dopaminergic drugs. The dopamine activity in the caudate and accumbens nuclei appears to be involved in the control of voluntary movement and locomotion.[35] Noradrenergic neurons seem to be involved in the regulation of attention, arousal and sleep-awake cycles as well as learning and memory,

Table I. Location and physiological function of serotonin 5-HT receptor subtypes commonly manipulated in exercise physiology (reproduced from Cooper et al.,[14] by permission of Oxford University Press, Inc.)

Subtype	High-density regions	Function
5-HT$_{1A}$	Raphe nuclei, hippocampus	Hyperpolarisation reduces serotonin secretory releasing frequency. The autoreceptors regulate serotonin concentration in the synaptic cleft and consequently the extent of stimulation of the postsynaptic 5-HT receptors Postsynaptic 5-HT$_{1A}$ receptors are thought to be involved in thermoregulation, hypotension and sexual behaviour
5-HT$_{1B}$	Substantia nigra, globus pallidus	Do not close or open ion channels, instead coupled to the adenylyl cyclase signal transduction pathway Through interaction with serotonin transporter, 5-HT$_{1B}$ receptors modulate serotonin release. The autoreceptors function is similar to the 5-HT$_{1A}$ receptor
5-HT$_{1D}$	Globus pallidus, substantia nigra, basal ganglia	Do not close or open ion channels, instead coupled to the adenylyl cyclase signal transduction pathway Through interaction with serotonin transporter, 5-HT$_{1B}$ receptors modulate serotonin release. The autoreceptors function is similar to the 5-HT$_{1A}$ receptor
5-HT$_{2A}$	Cortex, hippocampus, facial motor neurons	Opens or closes K$^+$ channels, activates several protein kinases and signal transduction through phosphoinositide hydrolysis 5-HT$_2$ receptor sensitivity is physiologically higher than that of pre- or postsynaptic 5-HT$_1$ receptors 5-HT$_2$ receptors have been associated with mood regulation and hallucinogenic activity
5-HT$_{2C}$	Widely distributed throughout the CNS	Opens or closes K$^+$ channels, activates several protein kinases and signal transduction through phosphoinositide hydrolysis 5-HT$_{2C}$ receptors have been associated with mood regulation, energy balance and hallucinogenic activity 5-HT$_{2C}$ receptors are involved in the control of the activity of the central dopaminergic system
5-HT$_3$	Peripheral neurons, entorhinal cortex, area postrema	Supports 5-HT$_2$ receptor activation by concomitant membrane depolarisation Suppression of 5-HT$_3$ receptor activity may contribute to the action of antidepressants

anxiety, pain, mood and brain metabolism.[36] In a similar manner to the dopaminergic system, noradrenergic mechanisms are involved in feelings of reward. Noradrenaline has also been implicated in aetiology of depression.

Based on these observations, Davis and Bailey[31] developed Newsholme's original hypothesis. Rather than the proposition that central fatigue was exclusively mediated through changes in the synthesis and metabolism of serotonin, the findings of this series of studies led to the suggestion that both serotonin and dopamine were important to the fatigue process. This revised central fatigue hypothesis suggests that an increase in the brain content ratio of serotonin to dopamine is associated with feelings of tiredness and lethargy, accelerating the onset of fatigue, whereas a low ratio favours improved performance through the maintenance of motivation and arousal. Given the strong relationship between alterations in neurotransmitters and neuromodulators and an individual's mood, it is likely that the sense of effort and its relationship with the willingness to start and continue exercise can be significantly influenced by the CNS.[2,8,31,37,38]

2. Central Fatigue and Serotonin: the Evidence

Much of the attraction of the hypothesis described by Newsholme et al.[8] was the potential for nutritional manipulation of neurotransmitter precursors to delay the onset of central fatigue and potentially enhance performance. In recent years, a number of studies have attempted to attenuate the increase in central serotonin levels through dietary supplementation with specific nutrients, including amino acids and carbohydrate (CHO). Other studies have employed pharmacological manipulations to alter the central extracellular neurotransmitter concentrations. Table II and table III give an overview of the human and animal studies that have attempted to manipulate brain neurotransmission through nutritional supplementation with amino acids or CHO

Table II. Effect of nutritional and pharmacological manipulation of central neurotransmission on exercise in humans

Study	Subjects	Manipulation	Protocol	Outcome
Segura and Ventura[39]	12 M	L-TRP	T_{exh} 80% $\dot{V}O_{2max}$	↑ T_{ex} ↔ RPE, HR, $\dot{V}O_2$
Blomstrand et al.[40]	107 M (BCAA) 111 M (placebo)	BCAA	30km cross-country or 42.2km marathon	↑ Exercise perf in slower runners ↔ Exercise perf in faster runners ↑ Mental perf
Stensrud et al.[41]	49 M	L-TRP	T_{exh} 100% $\dot{V}O_{2max}$	↔ T_{ex}
Wilson and Maughan[42]	7 M	Paroxetine (SSRI)	T_{exh} 70% $\dot{V}O_{2max}$	↓ T_{ex} ↔ RPE, HR, $\dot{V}O_2$, T_{core}
Davis et al.[43]	8 M	CHO	68% $\dot{V}O_{2max}$ up to 255 min	↑ T_{ex} ↓ f-TRP : BCAA
Davis et al.[44]	6 M	Fluoxetine (SSRI)	T_{exh} 70% $\dot{V}O_{2max}$	↓ T_{ex}
Hassmen et al.[45]	52 M	BCAA + CHO	30km cross-country	↔ Exercise perf ↑ Cognitive perf
Varnier et al.[46]	6 M	BCAA	Incremental T_{exh}	↔ T_{ex}
Pannier et al.[47]	8 M	Pizotifen (5-HT$_{2C}$ antagonist)	T_{exh} 70% $\dot{V}O_{2max}$	↔ T_{ex}
Blomstrand et al.[48]	5 M	BCAA or BCAA + CHO	T_{exh} 75% $\dot{V}O_{2max}$	↔ T_{ex}
Alves et al.[49]	8 M	TRP or TRP + CAF	T_{exh} 80% W_{max}	↔ T_{ex}, HR, RPE, $\dot{V}O_2$, T_{core}
van Hall et al.[27]	10 M	BCAA or TRP	T_{exh} 70–75% W_{max}	↔ T_{ex}
Madsen et al.[50]	8 M	BCAA	100km TT (cycling)	↔ TT perf, BCAA ↑ NH_3
Marvin et al.[51]	13 M	Buspirone (5-HT$_{1A}$ agonist)	T_{exh} 80% $\dot{V}O_{2max}$	↔ T_{ex}
Meeusen et al.[52]	7 M	L-DOPA (D precursor) Ritanserin (5-HT$_{2A/2C}$ antagonist)	T_{exh} 65% $\dot{V}O_{2max}$	↔ T_{ex}, HR, NH_3
Blomstrand et al.[53]	7 M	BCAA	60 min 70% W_{max}, 20 min 100% W_{max}	↓ RPE ↑ mental perf
Mittleman et al.[54]	7 M, 6 F	BCAA	T_{exh} 40% $\dot{V}O_{2peak}$ at 34°C	↑ T_{ex} ↔ T_{core}, mental perf
Davis et al.[55]	8 M	CHO or CHO + BCAA	Intermittent shuttle running to fatigue	↑ T_{ex}, CHO/CHO + BCAA vs placebo ↔ T_{ex}, CHO vs CHO + BCAA
Struder et al.[56]	10 M	BCAA, TYR, paroxetine (SSRI)	T_{exh}	↔ T_{ex} with BCAA, TYR ↓ T_{ex} SSRI
Meeusen et al.[57]	8 M	Fluoxetine (SSRI)	Cycle TT (~90 min)	↔ TT perf, RPE, PRL
Parise et al.[58]	11 M	Acute fluoxetine (SSRI)	Wingate and 80% $\dot{V}O_{2max}$ to fatigue	↔ T_{ex}
	12 M	Chronic fluoxetine (SSRI)	Wingate and 90% $\dot{V}O_{2max}$ to fatigue	↔ T_{ex}

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Table II. Contd

Study	Subjects	Manipulation	Protocol	Outcome
Piacentini et al.[59]	7 M	Venlafaxine (serotonin/NA agonist)	Cycle TT (~90 min)	↔ TT perf, RPE, HR ↑ ACTH, NA
Piacentini et al.[60]	7 M	Reboxetine (NA agonist)	Cycle TT (~90 min)	↔ TT perf, RPE, HR ↑ PRL, β-END, NA, ACTH
Sgherza et al.[61]	13 M, 5 F	Naloxolone (opioid antagonist)	Incremental T_{exh}	↓ T_{ex} ↑ RPE, HR
Piacentini et al.[62]	8 M	Bupropion (D/NA agonist)	Cycle TT (~90 min)	↔ TT perf, RPE, HR ↑ PRL, ACTH, NA
Bridge et al.[63]	12 M	Buspirone (5-HT$_{1A}$ agonist + D$_2$ antagonist) or Buspirone + pindolol (5-HT$_{1A}$ antagonist)	T_{exh} 73% $\dot{V}O_{2max}$ at 35°C	Positive relationship between TTE and non-serotonin component
Nybo[64]	8 M	CHO	180 min cycle 2 min maximum contraction	↑ voluntary activation ↓ RPE
Jacobs and Bell[65]	15 M	Modafinil (α$_1$ adrenergic agonist)	T_{exh} 85% $\dot{V}O_{2max}$	↑ T_{ex} ↓ RPE
Strachan et al.[66]	8 M	Paroxetine (SSRI)	T_{exh} 60% $\dot{V}O_{2max}$ at 32°C	↔ T_{ex}, PRL, cortisol
Cheuvront et al.[67]	7 M	CHO + BCAA	60 min 50% $\dot{V}O_{2max}$ + 30 min TT at 40°C	↔ TT perf, HR, T_{core}, mental perf
Watson et al.[68]	8 M	BCAA	T_{exh} 50% $\dot{V}O_{2max}$ at 30°C	↔ T_{ex}, T_{core}, HR, RPE ↑ NH$_3$
Strachan et al.[69]	6 M, 1 F	Pizotifen (5-HT$_{2C}$ antagonist)	40km TT at 35.5°C	↔ TT perf ↑ T_{core}
Winnick et al.[70]	10 M, 10 F	CHO	Intermittent shuttle running to fatigue	↑ T_{ex}, cognitive perf
Watson et al.[71]	9 M	Bupropion (D/NA agonist)	60 min 55% W_{max} + TT at 18 and 30°C	I: ↔ TT perf II: ↑ TT perf, T_{core}, HR ↔ RPE, thermal comfort
Blomstrand et al.[72]	5 M	CHO	180 min 200 ± 7W	↓ net cerebral TRP uptake

ACTH = adrenocorticotropin hormone; **β-END** = β-endorphin; **BCAA** = branched-chain amino acids; **CAF** = caffein; **CHO** = carbohydrates; **D** = dopamine; **F** = females; **f-TRP** = free tryptophan; **HR** = heart rate; **L-DOPA** = L-dihydroxyphenylalanine; **L-TRP** = L-tryptophan; **M** = males; **NA** = noradrenaline; **NH$_3$** = ammonia; **perf** = performance; **PRL** = prolactin; **RPE** = ratings of perceived exertion; **SSRI** = selective serotonin reuptake inhibitor; **T_{core}** = core temperature; **T_{ex}** = exercise time; **T_{exh}** = time to exhaustion; **TRP** = tryptophan; **TT** = time trial; **TTE** = run time to exhaustion; **TYR** = tyrosine; **$\dot{V}O_2$** = oxygen uptake; **$\dot{V}O_{2max}$** = maximal oxygen uptake; **$\dot{V}O_{2peak}$** = peak oxygen uptake; **W_{max}** = maximal wattage; ↓ indicates decrease; ↑ indicates increase; ↔ indicates no effect.

Table III. Effect of nutritional and pharmacological manipulation of central neurotransmission on exercise in animals

Study	Animals	Manipulation	Protocol	Outcome
Jacobs and Eubanks[73]	64 S-D rats + controls	Serotonin 5-HTTP inj. IP-SC	Tilt-cage and 3h activity monitored	$\downarrow$ Activity in dose-dependent manner
Gerald[33]	10 S-D rats/group	Amphetamine (D agonist)	T_{exh}	$\uparrow T_{ex}$ in dose-dependent manner
Francesconi and Mager[74]	24 S-D rats	Chlorpromazine inj. I-V (tranquilliser) L-TRP inj. I-V	T_{exh} (9.14 m/min) at 35°C	Chlorpromazine and L-TRP: $\uparrow T_{ex}$ and produced hypothermia
Heyes et al.[6]	57 S-D rats	6-OHDA lesion Apomorphine (D agonist) Clonidine (α_2-adrenergic agonist)	T_{exh} (36 m/min)	6-OHDA: $\downarrow T_{ex}$ Apomorphine: $\uparrow T_{ex}$ Clonidine: $\leftrightarrow T_{ex}$
Chaouloff et al.[5]	5 Wistar rats/group	Amphetamine (D agonist) Pargyline (MAO-B inhibitor) Alpha-methyl-p-tyrosine (catecholamine inhibitor) Haloperidol (D antagonist)	60 min treadmill ex (20 m/min, 5% grade)	Amphetamine: $\downarrow$ brain serotonin Pargyline: $\uparrow$ brain DOPAC Alpha-methyl-p-tyrosine: $\uparrow$ brain serotonin Haloperidol: no effect
Chaouloff et al.[75]	6–7 Wistar rats/group	NSD 1015 (serotonin 5-HT receptor antagonist) TRP	90 min ex (20 m/min)	NSD 1015: serotonin synthesis impaired in hippocampus
Hillegaart et al.[76]	Rats	8-OHDPAT (5-HT$_{1A}$ agonist) inj. SC	Open field activity and treadmill running	No effect
Wilckens et al.[77]	Wistar rats	TFMPP (5-HT antagonist) mCPP (5-HT$_{1C}$ agonist) DOI (5-HT$_2$ agonist) QD (5-HT agonist)	Spontaneous running wheel activity	mCPP: $\downarrow$ activity 5-HT$_{1C}$ Effect abolished by antagonist
Bailey et al.[78]	8 Wistar rats	mCPP (5-HT$_{1C}$ agonist)	T_{exh} (20 m/min, 5% grade)	$\downarrow T_{ex}$ in dose-dependent manner
Bailey et al.[32]	36 Wistar rats	QD (5-HT agonist) LY 53857 (5-HT receptor antagonist)	T_{exh} (20 m/min, 5% grade)	QD: $\downarrow T_{ex}$ in dose-dependent manner LY: $\uparrow T_{ex}$ at highest dose (1.5 mg/kg)
Bailey et al.[79]	8 Wistar rats/group	QD LY 53857	T_{exh} (20 m/min, 5% grade)	QD (1 mg/kg): $\downarrow T_{ex}$ by 32% LY (1.5 mg/kg): $\uparrow T_{ex}$ by 26%
Verger et al.[80]	34 Wistar rats	BCAA or CHO	T_{exh} (16 m/min, 5% grade)	BCAA: $\leftrightarrow T_{ex}$ CHO: $\uparrow T_{ex}$
Meeusen et al.[81]	Wistar rats	TRP	60 min ex (12 m/min)	$\uparrow$ serotonin in hippocampus No effect on fatigue
Calders et al.[82]	6 Wistar rats	BCAA inj. IP	T_{exh} (20 m/min 8% grade)	$\uparrow T_{ex}$, blood NH$_3$
Farris et al.[83]	7 horses	L-TRP or L-TRP/CHO	T_{exh} 50% $\dot{V}O_{2max}$	$\downarrow T_{ex}$ with L-TRP and L-TRP/CHO $\uparrow$ PRL
Calders et al.[84]	6 Wistar rats	BCAA inj. IP CHO inj. IP CHO + BCAA inj. IP	T_{exh} (20 m/min 8% grade)	$\uparrow T_{ex}$ with BCAA, CHO, CHO + BCAA $\leftrightarrow T_{ex}$ with saline
Connor et al.[85]	Rats	Reboxetine (NA agonist)	Forced swim test	Dose-dependent attenuation of the swim test induced increases in serotonin + D activity

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Table III. Contd

Study	Animals	Manipulation	Protocol	Outcome
Gomez-Merino et al.[86]	7 Wistar rats	L-valine inj. I-V	60 min ex (25 m/min)	↑ hippocampus serotonin with ex; ↔ serotonin with L-valine
Kalinski et al.[87]	15 CD-1 mice	Methamphetamine (D uptake inhibitor) inj. IP	T_{exh}	↓ T_{ex}, striatal D, DOPAC
Smriga et al.[88]	27 Wistar rats	BCAA + glutamine + arginine	Activity wheel + treadmill running (60 min at 5 and 25 m/min)	Positive relationship between running distance and BCAA-based solution preference
Rodrigues et al.[89]	8 Wistar rats	Physostigmine (acetylcholinesterase inhibitor) inj. I-V	T_{exh} (20 m/min 5% grade)	↔ T_{ex}; ↓ HR, heat storage
Soares et al.[90]	6 Wistar rats	TRP inj. I-V	T_{exh} (18 m/min 5% grade)	↓ T_{ex}
Hasegawa et al.[91]	12 Wistar rats	Tetrodotoxin (sodium channel blocker) perfusion into the PO/AH	120 min ex (10 m/min)	↓ heat loss; ↑ heat production, T_{core}; ↔ ex behaviour

5-HT = 5-hydroxytryptamine (serotonin); **5-HTTP** = 5-hydroxytryptophan; **6-OHDA** = 6-hydroxydopamine; **8-OHDPAT** = 5-HT$_{1A}$ agonist; **BCAA** = branched chain amino acids; **CHO** = carbohydrates; **D** = dopamine; **DOI** = 5-HT$_2$ agonist; **DOPAC** = 3,4-dihydroxyphenylacetic acid; **ex** = exercise; **HR** = heart rate; **inj.** = injection; **IP** = intraperitoneal; **I-V** = intraventricular; **L-TRP** = L-tryptophan; **LY 53857** = 5-HT antagonist; **mCPP** = 5-HT$_{1C}$ agonist; **NA** = noradrenaline; **NSD 1015** = 5-HT antagonist; **PO/AH** = preoptic area and anterior hypothalamus; **PRL** = prolactin; **QD** = quipazine dimaleate; **SC** = subcutaneous; **S-D** = Sprague-Dawley; **T$_{core}$** = core temperature; **T$_{ex}$** = exercise time; **T$_{exh}$** = time to exhaustion; **TFMPP** = 5-HT antagonist; **TRP** = tryptophan; **VO$_{2max}$** = maximal oxygen uptake; ↓ indicates decrease; ↑ indicates increase; ↔ indicates no effect.

or by the administration of pharmacological agents that are known to act directly on the CNS.

2.1 Nutritional Manipulation of Neurotransmission: Branched-Chain Amino Acid, Tryptophan and Carbohydrate Supplementation

As TRP competes with BCAA for transport across the BBB into the CNS,[15] reducing the plasma concentration ratio of f-TRP to BCAA through the ingestion of exogenous BCAA has been suggested as a practice to attenuate the development of central fatigue. The first investigation undertaken to test the efficacy of BCAA supplementation at attenuating serotonin-mediated fatigue was a field study of the physical and mental performance of male volunteers competing in either a marathon or a 30km cross-country race.[40] The findings suggested that both physical (race time) and mental (colour and word tests) performance were enhanced in those receiving BCAA prior to exercise. However, enhanced exercise performance was only witnessed in subjects completing the marathon in times slower than 3 hours 5 minutes. The authors suggested the faster runners may have developed an increased resistance to the feelings associated with central and peripheral fatigue as a result of their training. It is worth noting that this was a field-based study, and as such has a degree of ecological validity, but the findings may be limited due to a lack of experimental control.

While there is some additional evidence of BCAA ingestion influencing ratings of perceived exertion (RPE)[53] and mental performance,[45,92] the results of several apparently well controlled laboratory studies have not demonstrated a positive effect on exercise capacity or performance. No ergogenic benefit has been reported during prolonged fixed intensity exercise to exhaustion,[27,48,53,56] prolonged time trial performance,[45,50] incremental exercise[46] or intermittent shuttle-running.[55] Given the clear association between cognitive function/performance and brain neurotransmission, it seems unusual that few studies have attempted to evaluate the effects of exercise and central fatigue on mental performance.

Work conducted by Mittleman et al.[54] has provided some support for BCAA and the apparent role of serotonin in the fatigue process. A 14% increase in capacity to perform low intensity (40% maximal oxygen uptake [$\dot{V}O_{2max}$]) exercise was reported following BCAA supplementation when compared with a polydextrose placebo. No difference in peripheral markers of fatigue was reported between the two exercise bouts. The authors concluded that the supplementation regimen was successful in limiting the entry of TRP into the CNS, attenuating serotonin-mediated fatigue. However, the unique aspect of this study was that the trials were undertaken in a warm environment (34.4°C), with subjects seated at rest for 2 hours before exercise in these conditions. BCAA supplementation began 60 minutes prior the start of exercise, resulting in a 2- to 3-fold reduction in the plasma concentration ratio of f-TRP to BCAA. In contrast, two subsequent studies have failed to support an effect of BCAA supplementation on exercise capacity in the heat.[67,68] The role of the CNS in the development of fatigue during prolonged exercise in a warm environment is discussed in section 6.

These conflicting findings question whether the attenuation of TRP uptake through the provision of exogenous BCAA can significantly influence central neurotransmission, but such effects may be influenced by the exercise and supplementation protocol employed as well as the group of subjects studied. These methodological differences make comparisons between studies difficult. In particular, incremental or high-intensity protocols may not be ideal tests, due to the relatively short duration of exercise and co-ingestion with CHO may mask any potential ergogenic benefit (see section 5). Additionally, a time delay is thought to exist between changes in peripheral amino acid availability and the cerebral uptake of f-TRP,[93] meaning that the pre-exercise ingestion period employed in some investigations may have been insufficient to alter brain neurotransmission significantly during the protocol studied.

While support for a benefit of BCAA ingestion in humans is limited, particularly when exercise is performed under temperate conditions, the response appears to be different in animals, where a clear increase in exercise capacity[82,84] and free running activity[88] has been shown. A study conducted by Verger et al.[80] observed no difference in time to exhaustion following BCAA ingestion in rats when compared with a placebo condition, but it is not clear why these results fail to agree with those of Calders et al.[82,84] The development of *in vivo* brain microdialysis has enabled the direct analyses of extracellular neurotransmitters and metabolites from the brain of resting and active animals with limited tissue trauma. Meeusen et al.[81] demonstrated that increased TRP availability resulted in an elevation in extracellular serotonin and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in 24-hour fasted rats. When TRP was administered prior to 60 minutes of treadmill running, the exercise serotonin response was amplified. Surprisingly, although the extracellular serotonin concentration increased by >100%, there were no signs of early fatigue, with all animals able to finish the total running session. Evidence for the role of BCAA in limiting TRP entry into the CNS and attenuating the increase in serotonin has been reported in rodents using *in vivo* brain microdialysis.[86] During the placebo trial (saline infusion), a progressive increase in extracellular serotonin was apparent in the hippocampus as exercise continued, but this elevation was abolished when exercise was preceded by a peripheral infusion of valine.

While there are reports of TRP supplementation producing a marked reduction in the exercise capacity of horses[83] and rodents,[90] Segura and Ventura[39] reported a 49% increase in time to exhaustion following L-TRP supplementation. The authors of this study hypothesised that administration of L-TRP before exercise could contribute to a decreased sense of discomfort and pain associated with prolonged exercise, but these data are confounded by the spectacular improvement of two of the eight subjects (160% and 260% increase in running time). Subsequent work investigating the ingestion of between 1.2 and 3.0g of TRP immediately prior to the start of exercise produced no effect on exercise capacity in human subjects.[27,41,49] In particular,

Stensrud et al.[41] failed to demonstrate a change in exercise capacity following 24 hours of tryptophan supplementation. The findings of this study appear particularly convincing due to the unusually large sample size studied (49 subjects), although the intensity of exercise was high (100% $\dot{V}O_{2max}$) and this may have limited the efficacy of the treatment.

Although the ratio between TRP and BCAA may be changed by oral supplementation of BCAA or TRP, the net effect on the brain is not yet clear. This was demonstrated by Nybo et al.[94] who evaluated the cerebral balances of dopamine, tyrosine and TRP, as well as the cerebral oxygen-to-CHO uptake ratio during prolonged exercise with a normal or elevated core temperature. They reported a positive relationship between cerebral TRP balance and the arterial TRP concentration and this was a better predictor of TRP uptake than the plasma concentration ratio of f-TRP to BCAA. However, there was only a net uptake of TRP by the brain in half the subjects tested, and this response was not influenced by body temperature. Failure to observe any increase in cerebral TRP uptake when hyperthermia does not support a significant role of serotonin in the development of central fatigue in the heat. The importance of central fatigue during prolonged exercise in a warm environment is discussed in section 6.

It appears that there is limited or only circumstantial evidence to suggest that exercise performance in temperate conditions can be altered by nutritional manipulation through TRP or BCAA supplementation. Another nutritional strategy that may influence serotonin synthesis, and potentially the development of central fatigue, is CHO feeding. The ingestion of CHO suppresses lipolysis, lowering the circulating concentration of plasma FFA and consequently limiting the exercise-induced rise in f-TRP. Recognising this, Davis et al.[43] suggested CHO ingestion as a means of reducing cerebral TRP uptake. A 5- to 7-fold increase in the plasma concentration ratio of f-TRP to BCAA was reported under placebo conditions. Supplementation with a 6% or 12% CHO solution attenuated the increase in plasma FFA and f-TRP, reducing the plasma concentration ratio of f-

TRP to BCAA in a dose-dependent manner. Exercise capacity during CHO trials was increased over the placebo, suggesting CHO ingestion as an effective means of delaying the onset of central fatigue, but it is difficult to interpret the contribution of central factors from the widely reported benefits of CHO at attenuating peripheral fatigue. Recent work has demonstrated that CHO ingestion during exercise attenuated the cerebral uptake of TRP as well as preventing the development of hypoglycaemia, supporting these initial findings.[72] The importance of CHO availability to the CNS is discussed in detail in section 5.

2.2 Pharmacological Manipulation of Neurotransmission: Selective Serotonin Reuptake Inhibitors

Manipulation of central neurotransmission through the administration of pharmacological agents dates back to the 19th century, where opium and bromides were commonly used. The introduction of antipsychotics for the treatment of schizophrenia and significantly, fluoxetine (Prozac™),[1] started the widespread trend for the prescription of drugs acting on the CNS for the treatment of depression. Today these drugs are used in the treatment of a number of psychiatric disorders (including anxiety disorders, obsessive compulsive disorder); consequently, the pharmaceutical industry is constantly producing novel, more selective agents. The worldwide prescribing rates for drugs of this nature have increased dramatically over the last decade. There has been a >2-fold increase in prescription rates for antidepressants alone reported in Australia, the UK and the US. This may be due to an increase in the spectrum of treatments available, improved recognition of mental ill health, and the pressure to prescribe drugs in favour of other treatments.

A series of studies by Bailey et al.[32,78] examined the effects of pharmacological manipulation of brain serotonin levels in rats through the administration of specific 5-HT receptor agonists and antagonists. This original work provided convincing evidence

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1 The use of trade names is for product identification purposes only and does not imply endorsement.

for a role of serotonin in the development of fatigue, with a dose-dependent reduction in exercise capacity reported when central serotonin activity was augmented by the acute administration of a general 5-HT receptor agonist.[78] Brain serotonin and dopamine content progressively increased during exercise, but at the point of exhaustion a marked fall in tissue dopamine content was apparent. Furthermore, exercise capacity was enhanced by a 5-HT receptor antagonist (LY-53857), although this was apparent only when the highest dose was administered.[32] These alterations to exercise capacity occurred without any change in core temperature, circulating metabolites or stress hormones.[79] Additionally, muscle glycogen concentrations at fatigue were higher when a 5-HT receptor agonist (quipazine dimaleate) was administered, suggesting that the accelerated fatigue did not occur as a result of limited glycogen availability. It is important to note that the highest doses of drugs given in these studies were many times greater than would normally be administered for therapeutic cases.

The first human studies designed to investigate the effects of pharmacological manipulation of central serotonin on exercise capacity employed a class of drugs known as selective serotonin reuptake inhibitors (SSRIs). SSRIs are agents that selectively inhibit the reuptake of serotonin into the presynaptic nerve terminal, thus prolonging its action. They increase the concentration of serotonin present at the postsynaptic receptors and have been widely administered in the treatment of various psychiatric disorders, in particular depression. Three studies have investigated the effects of an acute dose of paroxetine (Paxil™, Seroxat™): two reporting a decrease in time to exhaustion,[42,56] while a recent study did not detect any difference in exercise capacity when cycling at 60% $\dot{V}O_{2max}$ in 32°C.[66] Fluoxetine (Prozac™) produced a reduction in exercise capacity in subjects cycling at 70% $\dot{V}O_{2max}$ to exhaustion.[44] Meeusen et al.[57] demonstrated that performance of a 90-minute time trial at 65% maximal wattage (W_{max}) was not affected by fluoxetine, although some of the hormonal and metabolic responses to the drug observed were comparable with other SSRI studies. The neuromuscular and performance effects of short- and long-term exposure to fluoxetine have also been examined.[58] Serotonin has been demonstrated to alter an individuals' sensation of pain, and this study differs from many others in this area by investigating whether manipulation of serotonergic neurotransmission could alter the response to high-intensity and resistance exercise. However, short- and long-term SSRI intake failed to influence force production during maximal voluntary contractions or high-intensity exercise performance.

Several possible factors such as exercise protocol, different dosages or drug utilisation might help explain the conflicting results observed. While there is some evidence that short-term SSRI ingestion can reduce an individual's capacity to perform prolonged exercise,[42,44,56] it has been suggested that this occurred due to a disturbance in regulatory homeostasis of the serotonin system, perhaps via a disruption in pre- or postsynaptic receptor function[95] rather than an increase in the activity of the serotonergic neurons. It was recently demonstrated with functional magnetic resonance imaging that several SSRIs, including paroxetine, can produce a dose-dependent activation of the entire motor pathway in a way that favours motor output.[96] This suggests that serotonin plays an important role in the initiation of movement and the continuation of motor behaviour.

2.3 Serotonin 5-HT Receptor Agonists or Antagonists

Serotonin produces its effects through a variety of membrane-bound receptors. Serotonin and its receptors are found both in the central and peripheral nervous system, as well as in a number of non-neuronal tissues in the gut, cardiovascular system and blood. 5-HT receptors are diverse and numerous and represent one of the most complex families of neurotransmitter receptors. Over the past decade, >14 different 5-HT receptors have been cloned through molecular biological techniques. These studies have helped to identify new therapeutic targets, and aided an understanding of the multiple

roles played by serotonin in the brain. Overall, seven distinct families of 5-HT receptors ($5\text{-HT}_{1\text{-}7}$) have been identified, with as many as five within a given family. Only one of the 5-HT receptors is a ligand-gated ion channel; the other six belong to the G protein-coupled receptor family. In line with the studies that used SSRIs to manipulate central serotonergic activity, there have been several studies that used selective 5-HT agonists and/or antagonists during prolonged exercise to fatigue. According to the central fatigue hypothesis, 5-HT agonists would be expected to reduce exercise time to exhaustion, while 5-HT antagonists would imply an increase in exercise capacity.

Pannier et al.[47] investigated the effect of the 5-HT receptor antagonist pizotifen on endurance performance during treadmill exercise in humans. Pizotifen administration did not alter exercise time to exhaustion in this study, with similar findings reported in a recent investigation when performance was measured on a 40km time trial in 35°C.[69] Additionally, a specific centrally acting $5\text{-HT}_{2A/2C}$ antagonist did not influence performance on a bicycle trial to exhaustion at 65% W_{max}.[52] Taken together, these studies indicate that although one would expect 5-HT antagonism to positively influence performance, no difference was found neither on time trial performance or exercise time to exhaustion. Marvin et al.[51] exercised subjects at 80% $\dot{V}O_{2max}$ following oral administration of either placebo or the partial 5-HT_{1A} agonist buspirone. Ratings of perceived exertion were higher following buspirone and time to volitional fatigue fell significantly by approximately one-third following buspirone. This study supports the possible central modulation of exercise tolerance by serotonergic pathways, although a role for dopamine cannot be excluded. While this drug is thought to primarily act as a 5-HT_{1A} receptor agonist, it also produces a limited dopaminergic D_2 antagonism,[63] which may have played a significant role in mediating this response.

2.4 Combined Reuptake Inhibitors

Because of the complexity of brain functioning and the contradictory results from the studies that tried to manipulate only serotonergic activity, it appears unlikely that a single neurotransmitter system is responsible for the central component of fatigue. In fact, alterations in serotonin, catecholamines, amino acid neurotransmitters (glutamate, GABA) and acetylcholine have all been implicated as possible mediators of central fatigue during exercise.[37] These neurotransmitters are known to play a role in arousal, mood, motivation, vigilance, anxiety and reward mechanisms, and could therefore, if adversely affected, impair performance. It is therefore necessary to explore the different transmitter systems and their effect on the neuroendocrine response to endurance exercise. Since these drugs are used in the treatment of a wide variety of psychiatric disorders, the pharmaceutical industry is constantly producing novel, with potential to act on one or more neurotransmitter, systems.

In a series of studies, we supplemented athletes with venlafaxine a combined serotonin/noradrenaline reuptake inhibitor,[59] reboxetine a noradrenaline reuptake inhibitor[60] and bupropion, a combined noradrenaline/dopamine reuptake inhibitor.[62] Athletes performed two exercise trials requiring the completion of a preset amount of work as quickly as possible (~90 minutes), in a double-blind randomised crossover design. None of the above-mentioned reuptake inhibitors influenced (either negatively or positively) exercise performance (figure 1). Each drug clearly altered central neurotransmission since different neuroendocrine effects were observed depending on the type of reuptake inhibitor administered, as illustrated in figure 2 for prolactin (PRL) and growth hormone. While serotonin is thought to induce PRL release, SSRI administration did not increase the PRL response to exercise. Additionally, the noradrenaline/dopamine reuptake inhibitor was not able to decrease PRL release. Moreover, it appears that noradrenaline has a significant enhancing effect on growth hormone concentrations. This brings us back to the possible interaction between neurotransmitters and their mutual influ-
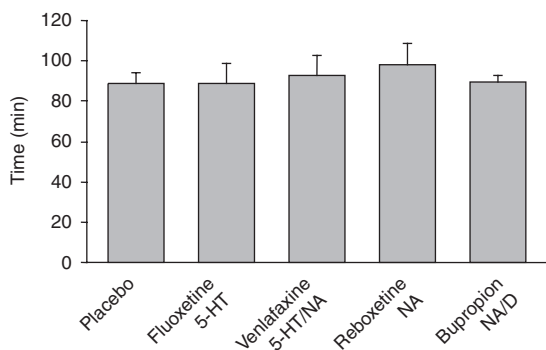
Fig. 1. Effects of different reuptake inhibitors on 90-minute time trial performance at an ambient temperature of 18°C.[57,59,60,62,97,98] **5-HT** = serotonin; **D** = dopamine; **NA** = noradrenaline.

ence on the hormonal response to a prolonged exercise protocol. Peripheral hormone concentrations have been employed as markers of central neurotransmitter activity and the application of these peripheral indices of neurotransmission is discussed in section 3.

2.5 Catecholaminergic Drugs

Dopamine and noradrenaline are neurotransmitters linked to the 'central' component of fatigue for their well known role on motivation and motor behaviour,[37,75] and are therefore thought to have an enhancing effect on performance. Early pharmacological manipulation of central neurotransmission to improve exercise performance focused largely on the effects of amphetamines, with studies of this nature undertaken by German scientists during the Second World War. Amphetamine is a close analogue of dopamine and noradrenaline, thought to act directly on catecholaminergic neurones to produce a marked elevation in extracellular dopamine concentrations. This response is believed to be mediated through the stimulation of dopamine release from storage vesicles, inhibition of dopamine reuptake and the inhibition of dopamine metabolism by monoamineoxidase.[14] Amphetamines may also limit the synthesis of serotonin through a reduction in TRP hydroxylase activity and a direct interaction between dopamine release and serotonergic neurotransmission.[5] Studies have demonstrated a clear

performance benefit following the administration of amphetamine to both rodents[33] and humans.[99,100] The ergogenic action of amphetamine is thought to be mediated through the maintenance of dopamine release late in exercise. These findings have led to the widespread use of amphetamines in many endurance events, with a long history of abuse in cycling events in particular.

Animal studies show that at the point of fatigue dopamine extracellular concentrations are low, possibly due to the interaction with brain serotonin,[78] or a depletion of central catecholamines.[31] The various studies that examined the influence of exercise on brain neurotransmitters indicate that both central dopaminergic and serotonergic activity are influenced by exercise. Chaouloff et al.[5] examined if compounds known to affect dopamine activity in the brain could modify the serotonin response in the brain during exercise. The dopamine metabolism was increased in serotonin-rich regions. Administration of amphetamine, while increasing levels of TRP in the brain, diminished the formation of 5-HIAA, suggesting that serotonergic activity was reduced. The relative inhibition of synthesis of serotonin induced by running was thus potentiated by administration of amphetamine while α-methyl-p-tyrosine (inhibitor of catecholamine synthesis) prevented this effect of exercise, and haloperidol (dopamine antagonist) did not produce any significant change. Further evidence for a role of dopamine in the development of central fatigue is provided by work conducted by Heyes et al.[6] Infusion of apomorphine (a dopamine agonist) has been shown to prolong and partially restore exercise capacity following destruction of dopaminergic neurons with 6-hydroxydopamine. Additionally, pre-exercise treatment with methamphetamine, which produces a depletion of striatal dopamine, resulted in a marked reduction in running time to exhaustion in rodents.[87]

Intracranial self-stimulation (ICSS) has been employed as a model to induce exercise in rodents, removing the need to administer aversive electric shocks.[34] ICSS involves the implantation of an electrode into the ventral tegmental area, the origin of the dopaminergic projection, which triggers elec-

trical stimulation to this area of the brain when the animal maintained a pre-determined running speed. The dopaminergic reward associated with ICSS has been reported to enable rats to run around 50% longer when compared with the use of electric shock,[34] while producing no effect on peripheral variables relating to cardiovascular, metabolic or thermoregulatory function.[101] Although studies investigating changes in central dopamine using electric shock grids to stimulate running should be viewed with caution as the stress associated with this form of motivation has been demonstrated to significantly increase dopamine release,[102] it needs to be pointed out that these studies clearly demonstrate the involvement of the dopaminergic system in increasing performance.

Despite the apparent link between exercise and catecholaminergic neurotransmission demonstrated in animals, there has been relatively little work conducted to assess the effects of dopamine and

noradrenaline manipulation on exercise capacity in humans. The administration of L-3,4-dihydroxyphenylalanine (L-DOPA), an intermediate in the catecholamine pathway, has been employed to effectively bypass the rate-limiting step in dopamine and noradrenaline synthesis. L-DOPA has been used in the management of Parkinson's disease, a disorder characterised by a loss of motor control and coordination, to maintain central dopamine neurotransmission. Meeusen et al.[52] examined the effect of L-DOPA on exercise capacity in endurance-trained males. Ingestion of L-DOPA 24 hours and immediately before commencing exercise had no effect on submaximal time to exhaustion or peripheral cardiovascular or metabolic measures during exercise.

The recent development of brain imaging technologies will help shed light on the effect of exercise on central neurotransmission, but the application of these techniques are still in their infancy. Wang et
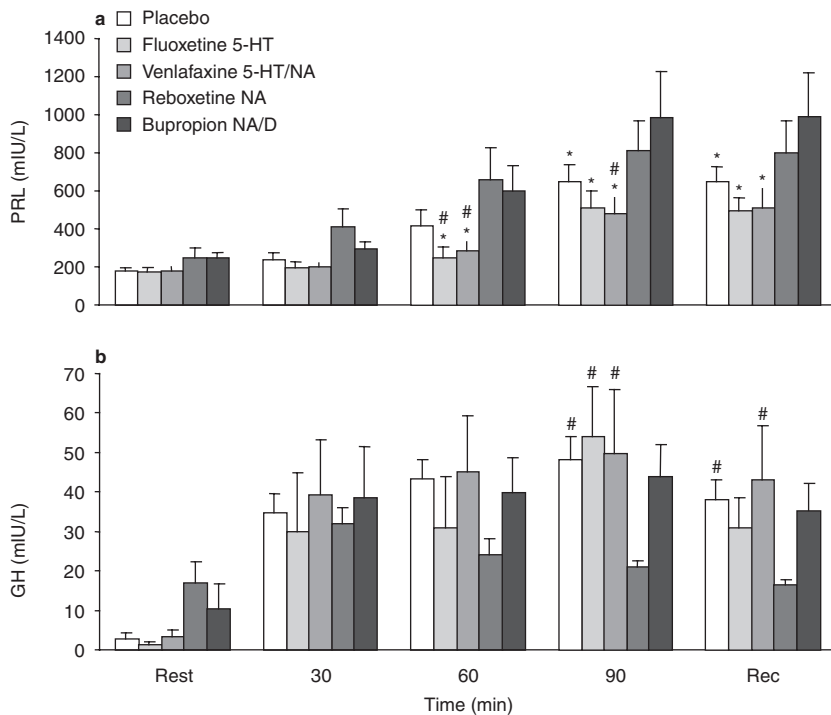

Fig. 2. Effects of a 90-minute time trial performance at an ambient temperature of 18°C on (**a**) the prolactin (PRL) concentration (mean ± SD) and (**b**) the growth hormone (GH) concentration (mean ± SD).[57,59,60,62,98] **5-HT** = serotonin; **D** = dopamine; **NA** = noradrenaline; **rec** = recovery; **#** indicates significantly ($p < 0.05$) different from reboxetine; * indicates significantly ($p < 0.05$) different from bupropion.

al.[103] evaluated the effects of exercise on striatal dopamine release in the human brain using positron emission tomography (PET) scans. They did not find significant changes in synaptic dopamine concentration during vigorous treadmill exercise for 30 minutes. They concluded that this level of exercise does not induce changes in striatal dopamine release that are large enough to be detected with the PET raclopride method. Thus it seems that experimental evidence for a relationship between fatigue and dopamine deficiency in the healthy human brain during prolonged exercise is lacking at present. While there is good indirect evidence that catecholaminergic neurotransmission plays an important role in the fatigue process, further evaluation of dopaminergic activity during exhaustive exercise is required before firm conclusions on the relevance of dopamine for central fatigue can be drawn.

3. The Use of Prolactin and Other Hormones as Peripheral Indices of Brain Neurotransmitter Activity

Although recent imaging techniques such as PET and single photon emission computed tomography scans are promising, it is difficult to directly determine changes in central neurotransmission in human subjects. While these methods are increasingly being employed in a clinical setting, their use is restricted in exercise physiology due to expense, access to experienced operators and logistical problems associated with performing exercise in or close to the equipment. Therefore, the measurement of changes in circulating concentrations of peripheral hormones has been employed as an index of central neurotransmission.[100]

The premise that circulating hormones may be used to determine changes in central neurotransmission is based on the role of central monoamines in the control of hormone release from the anterior and posterior pituitary gland. The paraventricular nucleus of hypothalamus is the major integrating link between the nervous and endocrine systems, with inputs from several areas of the brain governing the release of pituitary hormones through the infundibulum stalk. Changes in the activity of serotonin,

dopamine and noradrenaline neurons innervating the hypothalamus stimulates the synthesis of releasing and inhibiting hormones that act directly on areas of the pituitary to trigger the release of hormones into the peripheral circulation. Adrenocorticotropic hormone (ACTH) release from the pituitary also exhibits control over the adrenal gland, influencing cortisol release. This has led to the term the hypothalamic-pituitary-adrenal (HPA) axis.

While the hypothalamus receives input from a number of cerebral structures, it is clear that the stimulation of different receptors within the neuroendocrine control sites produces a differing action on hormone release.[104,105] The application of neuroendocrine tests to disturb central neurotransmission has provided insight into the relative contributions of serotonin, dopamine and noradrenaline in the regulation of pituitary hormone release. However there remains a degree of uncertainty regarding the coordination of the endocrine response particularly under conditions of stress, including exercise.[106]

There is a considerable body of evidence to indicate a role for several neurotransmitters in the regulation of PRL, ACTH, cortisol and growth hormone secretion.[104-106] The most important PRL inhibiting factor is dopamine, which acts on the two most potent PRL-releasing factors: thyrotropin-releasing hormone and oxytocin. While basal secretion of PRL is primarily regulated by tonic inhibition by dopamine, serotonergic regulation originates from cells in the dorsal raphe nucleus with activation of 5-HT$_{1A}$, 5-HT$_{2A/C}$, 5-HT$_3$ receptors stimulating the secretion of PRL.[105] The neurons producing PRL inhibiting and releasing factors receive serotonergic innervation ascending mainly through the median forebrain bundle from the dorsal raphe nucleus of the brain stem. These serotonergic pathways participate in regulation of PRL secretion during stress, pregnancy and lactation.[107]

Many studies in exercise science have monitored changes in peripheral hormone concentrations as an indication of brain neurotransmission (hormones such as PRL and ACTH). While BCAA ingestion does not appear to influence PRL concentrations at

rest, except when large quantities (60g) are ingested,[108,109] early evidence demonstrated a clear relationship between serotonergic activity and plasma concentrations of PRL during exercise.[110,111] These findings resulted in the widespread use of PRL as a peripheral index of changes in central serotonergic activity during exercise. However nutritional and pharmacological manipulation of central serotonin activity have failed to alter the PRL response to exercise.[56,66,69] Recently, it has become clear that PRL release during activity is not governed solely by serotonin, but through a complex interaction between a number of neurotransmitter systems.[1] Evidence also suggests that elevated brain temperature may provide a stimulus for PRL release whilst at rest and during exercise.[112]

At the pituitary level, only a few substances play a role as primary neurohormones by robustly affecting hormone secretion (e.g. dopamine), while many others can act as modulators by amplifying or diminishing the effect of a primary neurohormone.[107] One of the major functions of and dopaminergic and GABAergic neurons is negative-feedback regulation of PRL secretion to prevent an exaggerated PRL output during specific physiological situations (e.g. lactation). It is generally assumed that excitatory amino acids exert their effects on PRL secretion by acting at hypothalamic targets. It has been reported, however, that glutamate increases PRL secretion. Other factors such as somatostatin, neuropeptide Y, galanin, substance P, endogenous opioids also play an important role in regulating PRL secretion. Furthermore, it seems quite conceivable that adrenergic modulation, mediated by either noradrenaline or adrenaline, plays an important role in stress-induced PRL secretion.

The interpretation of the hormone responses to agents that challenge serotonin, such as agonists, antagonists and precursors, in terms of specific receptor subtypes is by no means straightforward. Multiple receptor subtypes may contribute to a specific response if the agent is not specific, or if synergistic effects of receptor stimulation are involved. Furthermore, there are several neurotransmitter interactions that will occur, not only through direct interaction, but certainly also through different receptor subtypes that might have opposite effects depending on the situation on the neuron (pre- or post-synaptic; located at the cell bodies, or nerve endings etc.). Convincing evidence for a significant role of serotonin in governing PRL release during prolonged exercise is poor. Therefore, we advise our colleagues to consult the excellent review of Freeman et al.[107] on the regulation of PRL release before using this hormone as 'the' indicator of brain serotonergic activity.

4. Is 'What We See What We Get': is the Evidence for Central Fatigue that Straightforward?

It is known that serotonin and the other monoamines have been implicated in the aetiology of numerous disease states, including depression, anxiety, social phobia, schizophrenia, obsessive-compulsive and panic disorders; in addition to migraine, hypertension, pulmonary hypertension, eating disorders, vomiting and irritable bowel syndrome, all of which are driven by one or several of the receptor types. This also implies that assigning the central fatigue during prolonged exercise to a specific neurotransmitter or receptor is extremely unlikely. Further research is necessary to explore the complex functioning and interaction of the serotonergic and other neurotransmitter systems during exercise.

One should always be cautious when interpreting the results of studies that used pharmacological manipulations especially as dosages and drugs used often differ, in addition to differences in the exercise protocol employed. The equivocal findings of these pharmacological studies may be explained by the complexity of the actions of the drugs employed. In particular, these agents have varying affinities and specificities for the receptors they target. Furthermore, the metabolites of these drugs may have different effects on the reuptake sites, for example the metabolite of fluoxetine is as potent as the 'parent chemical', while paroxetine does not have a metabolite with any affinity for the site of action.[113]

Recent microdialysis experiments from our laboratory[97,98] have demonstrated an increase in extracellular hippocampal concentrations of noradrenaline and dopamine at a relatively low bupropion dose and an increase in noradrenaline and serotonin after venlafaxine administration. Although there is good evidence that acute doses of drugs influencing central neurotransmission can transiently influence neurotransmission in rodents,[32,97,98] the effect of these drugs on the human CNS is not clear at present. Evidence from clinical studies suggests that an acute administration of monoamine reuptake inhibitors in humans may produce little change in extracellular neurotransmitter concentrations, due to a reduction in cell firing rate caused by presynaptic autoreceptor-mediated feedback inhibition.[30] In the light of contrasting results produced between animal and human studies (e.g. BCAA supplementation), it is worth exercising a degree of caution when extrapolating the results of animal studies to humans. At present, it is not clear why these differing responses occur, but this may be potentially explained by differences in the homeostatic regulation of central neurotransmitter homeostasis between species.

5. Are There Other Possible Factors Responsible for Central Fatigue?

Fatigue, in particular central fatigue, is a complex and multifaceted phenomenon. There are several other possible cerebral factors that might limit exercise performance, all of them influencing signal transduction, since the brain cells 'communicate' through chemical substances. Not all of these relationships have been explored in detail, and the complexity of brain neurochemical interactions will probably make it very difficult to construct a single or simple statement that covers the central fatigue phenomenon.

Other neurotransmitters such as glutamate, acetylcholine, adenosine and GABA have been tentatively suggested to be involved with the development of central fatigue,[114-117] but there has been too few data published to date to draw any firm conclusions regarding their importance. Attention has also been given to the influence of ammonia (NH_3) on cerebral metabolism.[31,118,119]

During prolonged exercise, the plasma concentration of NH_3 increases, largely as a result of the deamination of BCAA, rather than the deamination of adenosine monophosphate to inosine monophosphate. This response appears to be amplified by reduced glycogen availability,[120,121] hyperthermia[122,123] and the ingestion of BCAA.[27,28,68] This increase in NH_3 production is one possible explanation for a failure to observe a positive effect of BCAA supplements on physical and mental performance, despite a good rationale for their use. Since NH_3 can readily cross the BBB, it may enter the CNS where excessive accumulation may have a profound effect on cerebral function. Evidence suggests that hyperammonaemia has a marked effect of cerebral blood flow, energy metabolism, astrocyte function, synaptic transmission and the regulation of various neurotransmitter systems.[124] Therefore, it has been considered that exercise-induced hyperammonaemia could be a mediator of CNS fatigue during prolonged exercise.[31,118] Some support for this hypothesis is obtained from experiments with rats,[117,125] but in humans the influence of exercise on the cerebral NH_3 responses have only been sparsely investigated.[2] Recently, Nybo and Secher[2] found that during prolonged exercise the cerebral uptake and accumulation of NH_3 may provoke fatigue, e.g. by affecting neurotransmitter metabolism. However, further investigations of exercise conditions associated with marked hyperammonaemia are wanted to determine if the cerebral NH_3 uptake during exercise in healthy humans may increase to the extent where it influences neurotransmission and motor performance.

In recent years, the role of central adenosine has been investigated through its association with caffeine. The ergogenic effect of caffeine was originally thought to be mediated through an increase in fat oxidation rate, thus sparing muscle glycogen.[126] Subsequent work has largely failed to provide convincing support for this mechanism, leading to the suggestion that the effects of caffeine supplementation are centrally mediated. Caffeine is a potent

adenosine antagonist that readily crosses the BBB, producing a marked reduction in central adenosine neurotransmission. Adenosine inhibits the release of many excitory neurotransmitters, including dopamine and noradrenaline, consequently reducing arousal and spontaneous behavioural activity. The central effect of caffeine has recently been demonstrated by Davis et al.,[116] with a marked increase in exercise capacity observed following an infusion of caffeine into the brain of rodents. Interestingly, a marked reduction in exercise capacity was apparent when an adenosine agonist was injected centrally and this response was attenuated when this drug was co-injected with caffeine.

Depletion of substrates within the CNS and/or alterations in the level of certain neurotransmitters are potential mechanisms underlying the decline in central activation during the sustained muscle contraction. Maintenance of blood glucose concentration is important for continuation of endurance exercise at a given exercise intensity. Supplementation of CHO results in a greater uptake of blood glucose by the exercising muscles, thereby preserving a high rate of CHO oxidation late in exercise when muscle glycogen levels are low.[127] The beneficial effect of glucose supplementation during prolonged exercise could also relate to increased (or maintained) substrate delivery for the brain. Exercise-induced hypoglycaemia has been reported to reduce brain glucose uptake and overall cerebral metabolic rate,[128] and is associated with a marked reduction in voluntary activation during sustained muscular contractions.[64] This reduction in CNS activation is abolished when euglycaemia was maintained. CHO ingestion also attenuated losses of mental function observed following high-intensity intermittent exercise, mimicking that encountered during many team sports.[70] Data from animal work suggest that glucose plays an important role in the regulation of central neurotransmission and alterations in extracellular glucose concentrations have been demonstrated to significantly influence serotonin release and reuptake during exercise and recovery.[29]

In addition to changes in circulating blood glucose, the possibility that the depletion of brain glycogen may be important to the development of fatigue during strenuous exercise has recently been explored. The store of glycogen found in the brain is typically overlooked due to its small size (0.5–1.5g), but as this is a dynamic store with a high rate of turnover, any disturbance could significantly influence neuronal function. A fall in the cerebral oxygen to CHO uptake ratio, determined using arterial-venous difference across the brain, has been reported following exhaustive exercise.[129] This suggests that glucose and lactate are being taken up by the brain in excess of oxygen, possibly to replenish brain glycogen stores or contribute to the *de novo* synthesis of neurotransmitters.[2] A reduction in brain glycogen has also been implicated in the homeostatic drive to sleep,[130] supporting a possible role in the fatigue process.

There is a growing body of evidence to support the idea that feelings of tiredness and fatigue during an illness can be triggered by the production of pro-inflammatory cytokines, in particular interleukins 1β (IL-1β) and 6 (IL-6).[131,132] Communication between the periphery and the brain through IL-1β has been termed 'sickness behaviour', and is thought to be a natural homeostatic reaction to promote recovery from infection by limiting non-essential activities. It is possible that a similar response occurs following bouts of strenuous exercise and recent data suggest that brain IL-1β is significantly elevated in several cerebral regions following downhill running in mice.[133] This central response was associated with a marked reduction in voluntary activity in the days following the muscle damaging exercise.

An energy-sensing role has been proposed for IL-6.[134] Increased muscle IL-6 production during exercise, modulated by the muscle glycogen content, has been shown to stimulate lipolysis and increase fat oxidation.[135] As IL-6 can readily cross the BBB, this may act as a negative feedback mechanism to the CNS contributing to the development of central fatigue.[136] An overproduction of IL-6 has also been implicated in unexplained underperformance syndrome, possibly through an effect on the CNS.[132] However, a number of studies have failed to find an association between central IL-6 release and feel-

ings of fatigue during prolonged exercise,[94,137-139] suggesting that IL-6 may not be a major mediator of fatigue under these conditions. The possibility that cytokines may play a role in fatigue is a relatively new idea, and further work is required to understand the relationship between exercise-induced cytokine production and the development of fatigue and/or recovery from strenuous exercise.

One area of the CNS that received little attention in relation to exercise is the BBB and the possibility that changes in its integrity may be involved in the fatigue process. The relative impermeability of the BBB helps to maintain a stable environment for the brain by regulating exchange between the CNS and the extra-cerebral environment. While the BBB is largely resistant to changes in permeability, there are situations where the function of the BBB may be compromised, including infections and fever, neuronal damage and hyperthermia.[140] Either short- or long-term changes in the permeability of this barrier may allow the entry or exit of species that can affect the metabolism of the brain and thus influence a wide range of homeostatic mechanisms.

There is some evidence that prolonged exercise may lead to increased BBB permeability. Animal studies have established that the BBB can be widely disrupted following 30 minutes of forced swimming exercise.[141,142] Additionally, a recent human study reported an increase in circulating serum S100β, a proposed peripheral marker of BBB permeability, following prolonged exercise in a warm environment. This response was not apparent when exercise was performed in temperate conditions.[143] At present, the functional consequences of changes in BBB permeability during exercise are not clear. A small increase in brain-blood interfacing during exercise may be desirable to facilitate the exchange of substrates and metabolites, such as glucose and lactate, and other substances into the CNS when cerebral blood flow is elevated. However, a marked change in BBB permeability during exercise may limit an individual's capacity to perform prolonged exercise by modifying the transport kinetics of neurotransmitter precursors and other metabolites or allowing the accumulation of unwanted substances in the CNS.

Endogenous opioids are released from the brain during prolonged exercise.[2] An abundance of work conducted during the 1980s focused on changes in circulating β-endorphin levels and mood, with the sensation known as 'runner's high' attributed to opioids.[144] Although the endogenous opioids have been 'attractive' as possible candidates to influence psychological aspects during exercise, conclusions are typically based on associations (or lack of correlation) between alterations in peripheral plasma β-endorphin and psycho-physiological factors. These data should be interpreted with caution.

Other cerebral metabolic, thermodynamic, circulatory and humoral responses could all lead to a disturbance of cerebral homeostasis and eventually central fatigue. We refer to the excellent review of Nybo and Secher[2] for detailed information. To date, there is evidence that because of the extreme disturbance of homeostasis that occurs during prolonged exercise, peripheral and central regulatory mechanisms will be stressed. However, for the moment it is not possible to determine the exact regulation and the importance of each factor.

6. Hyperthermia and Central Fatigue: Is There a Link with Brain Neurotransmitters?

6.1 Hyperthermia as a Possible Limiting Factor

The capacity to perform prolonged exercise is reduced in a warm environment. Galloway and Maughan[145] reported that the exercise capacity of non-heat acclimated males was greatest at 11°C, with a progressive fall in time to fatigue as ambient temperature was increased. Similar findings have been reported by subsequent work, with an inverse relationship reported between ambient temperature and exercise capacity.[146] Additionally, a 6.5% reduction in mean power output during a cycle time trial was reported when the ambient temperature was increased from 23°C to 32°C.[147] This response occurred despite little difference in rectal tempera-

ture between trials. Despite an understanding of the influence of ambient conditions on prolonged exercise capacity, the underlying mechanisms behind the deleterious effects of heat stress are not clear at present.

As impaired substrate availability or utilisation, accumulation of lactate or the progressive loss of body fluids do not adequately explain this reduction in performance when exercising in a warm environment, this has lead to the suggestion that the CNS may be important.[148,149] Fatigue during prolonged exercise in a warm environment may coincide with the attainment of a critical core temperature, suggesting that there may be a thermal limit to exercise performance. Nielsen et al.[150] first proposed this concept following a comprehensive investigation of the effects of the physiological adaptations associated with repeated exposure to the heat. Following a period of heat acclimation, exercise capacity was increased by 40%, with fatigue occurring at a similar oesophageal temperature of 39.7 ± 0.15°C on each occasion. The period of acclimation appeared to lower resting core temperature, allowing exercise time to be extended prior to the attainment of this 'critical' temperature. This concept has been supported by recent findings in humans[151] and rodents,[152] although Sawka et al.[13] observed that around 75% of individuals appear to fatigue at a rectal temperature of 39.1°C. Premature fatigue prior to the attainment of core body temperatures suggested as limiting by Nielsen et al.[150] may relate to the training status of the individual, with trained athletes seemingly able to push themselves to higher core temperatures. While it is currently unclear how an elevated body temperature contributes to the development of fatigue, it seems possible that a critical core temperature may serve as a protective mechanism preventing potential damage to the body's tissues by limiting further heat production.

Recent work suggests that hyperthermia may have a direct affect on the CNS.[137,138,153-157] Nielsen et al.[155] demonstrated that prolonged exercise in the heat was characterised by a progressive reduction in electroencephalogram activity from the prefrontal cortex, with an increase in the ratio of α to β frequency bands. This shift towards lower frequency α-bands is associated with feelings of tiredness and fatigue, and is observed during the transition from waking to sleep. In addition, a decline in cerebral blood flow has been reported during exercise with hyperthermia.[156] Perceived exertion is also significantly elevated by high core temperature compared with exercise at the same intensity under normothermic conditions.[138,153]

Whether exercise-induced hyperthermia influences neuromuscular function is not clear at present, with studies reporting a marked reduction[137] or little change[150,158] in force generation capacity. This discrepancy appears to relate to differences in the duration of contraction. Nybo and Nielsen[137] demonstrated that exercise-induced hyperthermia reduces the level of voluntary activation during a sustained maximal knee extension. The maximal contractions were performed immediately after bicycle exercise, which in the hyperthermic trial increased the core temperature to 40°C and exhausted the subjects after 50 minutes, whereas during the control trial the core temperature stabilised at ~38°C and exercise was maintained for 1 hour without exhausting the subjects. Of note, although the hyperthermic exercise trial exhausted the subjects, it did not impair the ability of the knee extensors to generate force, as signified by the similar force when electrical stimulation was superimposed. In addition, following a cycle protocol, force development during sustained handgrip contractions followed a similar pattern of response as for the knee extensors, indicating that the attenuated ability to activate the skeletal muscles did not depend on whether the muscle group had been active or inactive during the preceding exercise bout.[154]

Recent studies proposed that power output during self-paced exercise undertaken in warm ambient conditions may be determined by a mechanism of anticipatory regulation, relating to the avoidance of catastrophe rather than a critical limiting temperature. Tucker et al.[159] found that power output and integrated electromyographic activity of the quadriceps muscle began to decrease early during the self-paced exercise in the heat, but not in the cool trials

before the rectal temperature reached 40°C. They suggested that impaired exercise performance in the heat is not the result of a limiting core temperature, but occurs as part of the central regulation of skeletal muscle recruitment, which controls the rate of heat storage, thereby preventing the development of thermoregulatory derangement during self-paced exercise in the heat. Marino et al.[160] recently examined running performance and associated thermoregulatory responses of African and Caucasian runners in cool (15°C) and in hot (35°C) conditions during an 8km time trial. African runners ran faster only in the heat despite similar thermoregulatory responses as Caucasian runners, suggesting that the larger Caucasians reduce their running speed to ensure an optimal rate of heat storage without developing a critical limiting core temperature. An evolutionary perspective suggests that physiological safeguards should protect individuals before catastrophic hyperthermia,[161] and it seems likely that this is primarily a learned response developed through past experiences.[162] Although these studies demonstrate an 'alternative' central component in the fatigue process during hyperthermia, the underlying neurobiological mechanisms for these responses are not clear at present.

6.2 Is There a Link with Brain Neurotransmission?

Thermoreceptors in the preoptic anterior hypothalamus (PO/AH) are responsible for regulation of body temperature, with changes in body temperature detected through inputs from peripheral osmoreceptors and pressure receptors as well as the temperature of blood flowing to the brain. As serotonergic and catecholaminergic projections innervate areas of the hypothalamus, a change in the activity of these neurons may be expected to contribute to fatigue when core temperature is elevated.[38]

Although Mittleman et al.,[54] in a study supplementing subjects BCAA during exercise, stated that serotonin-mediated fatigue is important during exercise in the heat, two recent studies have failed to support these findings.[67,68] Cheuvront et al.[67] reported that BCAA, when combined with CHO, did not alter time trial performance, cognitive performance, mood, RPE, thermal comfort and rectal temperature in the heat when subjects are hypohydrated. In this study, hypohydration was used in order to increase plasma osmolality and accentuate the hyperthermia and cardiovascular strain. Additionally, ingestion of BCAA solution prior to, and during, prolonged exercise in glycogen-depleted subjects did not influence exercise capacity, rectal and skin temperature, heart rate, RPE and perceived thermal stress despite a 4-fold reduction in the plasma concentration ratio of f-TRP to BCAA.[68]

To date, there has been little investigation of the influence of pharmacological agents acting on the CNS on the response to prolonged exercise in a warm environment. Work conducted by Strachan et al.[66] recently investigated the effect of acute paroxetine (a SSRI) administration. While the drug induced a slight increase in core body temperature at rest and during exercise, time to exhaustion, perceived exertion and the hormonal response to exercise were not different between trials. Pitsiladis et al.[163] suggested that the high peripheral PRL levels observed during exercise in the heat could result from an increase in serotonergic neurotransmission during combined exercise and heat stress. As highlighted previously, one should be cautious when considering PRL as an outcome measure of central serotonergic activity. As evidence suggests that elevated brain and skin temperatures may provide a stimulus for PRL release during exercise,[112] this suggests that HPA-hormone secretion may be linked to the activity of the peripheral and central thermoreceptors, rather than changes in central serotonergic activity.

As there is limited evidence that high levels of dopaminergic activity is associated with an increased tolerance to exercise in the heat,[63] we recently examined the effect of a dual dopamine/noradrenaline reuptake inhibitor on performance in temperate (18°C) and warm (30°C) conditions.[71] Subjects were able to complete a pre-loaded time trial 9% quicker in the heat following an acute dose of bupropion, but this difference between treatments was not apparent in temperate conditions. An inter-

esting observation was that seven (of nine) subjects in the heat attained core temperatures ≥40°C in the bupropion trial, compared with only two during the placebo trial. In the light of these findings, it is possible to suggest that the action of this drug may dampen or override inhibitory signals arising from the CNS to cease exercise due to hyperthermia. Consequently, enabling individuals to maintain a high power output despite the attainment of high core temperatures. This response appeared to occur without any change in the subjects' perceived exertion or thermal sensation, and may potentially increase the risk of developing heat illness. Given the historical link between heat-related fatalities and amphetamine abuse in cycling (the case of Tom Simpson during the 1967 Tour de France, for example), manipulation of central catecholamines under conditions of heat stress may be a potentially dangerous practice. As evidence for a role of serotonin during exercise in the heat is limited[66-68] these data suggest that catecholaminergic neurotransmission may act as an important neurobiological mediator of fatigue under conditions of heat stress.

Serotonin and dopamine have been implicated in the control of thermoregulation at rest[164,165] and during exercise.[69,166] Pharmacologically induced increases in serotonergic activity have been demonstrated to transiently elevate core temperature in free-living rats, with the pattern of change in hypothalamic serotonin and 5-HIAA concentrations mirroring almost exactly the change in body temperature.[164] Strachan et al.[69] reported a marked elevation in core temperature at rest and during exercise following pizotifen (a 5-HT$_{2C}$ receptor antagonist) administration, suggesting a role for the 5-HT$_{2C}$ receptor in the regulation of core temperature.

Disturbances in cerebral neurotransmitter levels, especially serotonergic activity, have long been implicated in central fatigue, but little work has been reported examining whether hyperthermia alters serotonin levels in the brain.[161] Indeed, although many studies have shown that neurotransmission in the PO/AH, the exact role of each neurotransmitter is not established yet due to conflicting results probably related to experimental techniques or conditions such as the use of anaesthesia. Ishiwata et al.[167] attempted to clarify the role of serotonin in the PO/AH combining biotelemetry and microdialysis. They measured changes in core temperature and levels of extracellular serotonin and its metabolite 5-HIAA in the PO/AH during cold (5°C) and heat (35°C) exposure. They also perfused fluoxetine (SSRI) and 8-OH-DPAT (5-HT$_{1A}$ agonist) into the PO/AH. In both environmental conditions core temperature changed significantly, but no significant changes were noted in extracellular levels of serotonin and 5-HIAA. In addition, the perfusion of serotonin agents into the PO/AH did not affect core temperature despite the fact that serotonin in the PO/AH was increased or decreased. These results suggest that hypothalamic serotonin may not mediate acute changes in thermoregulation.

There is one study that investigated core temperature and neurotransmitters or their metabolites in the hypothalamus during exercise. Hasegawa et al.[166] reported that moderate exercise induced an increase in dopaminergic neural activity in the PO/AH and this was associated with a reduced body heat storage. However, the levels of serotonin and 5-HIAA did not change. This study suggest that temperature homeostasis during exercise depends on activation of the dopamine release in the PO/AH, not serotonin.

Recently, the functional role of PO/AH in thermoregulation during exercise has been investigated using *in vivo* brain microdialysis.[91] Tetrodotoxin (TTX), which blocks sodium channels, was perfused into the PO/AH to investigate whether this manipulation can modify thermoregulatory functions of exercising rats. The introduction of TTX into the PO/AH induced an increase in core temperature with a decrease in heat loss responses and an increase in heat production responses during exercise, suggesting that neurotransmission in the PO/AH region is involved in the regulation of body temperature, especially in heat dissipation. The exact role of the specific neurotransmitters needs to be clarified since noradrenaline and dopamine but not serotonin were influenced during these recent studies,[91,167] and a recent microdialysis study also have

suggested that the central cholinergic and GABA-ergic system might also be involved in thermoregulation during exercise.[89,168] Furthermore, until now there were few studies that focused on the relationship between thermoregulation, brain neurotransmission and exercise performance (central fatigue) in this field. Therefore, further research is necessary to elucidate the role of specific neurotransmitter systems on thermoregulation during exercise.

This was confirmed by a recent study where a dual dopamine/noradrenaline reuptake inhibitor (bupropion) was administered to rats while measuring brain, abdominal and tail temperatures.[91] The injection of bupropion immediately increased brain and abdominal temperature, while tail temperature decreased. This change in thermoregulation was accompanied by an increase in extracellular noradrenaline and dopamine release in the PO/AH, while no influence was shown on serotonin. The results of this recent study indicate that the catecholaminergic neurotransmission in the PO/AH is responsible for both heat accumulation and heat dissipation.

While many investigations have contributed to our understanding of the increased thermoregulatory and metabolic demands experienced during prolonged exercise in the heat, it is clear that the causes of fatigue are yet to be fully understood. While early work focused primarily on the increase in circulatory and thermoregulatory strain, there is now a body of evidence to suggest that central fatigue may be accelerated when exercise is performed in a warm environment.

7. Conclusions

Fatigue is determined by a complex interplay among many factors and it is often debated whether performance is limited by muscular, cardiovascular or central factors. Central fatigue is a form of fatigue that is associated with specific alterations of CNS functioning that may influence mood, the sensation of effort and tolerance to pain and discomfort. It may be strange to quantify the relative importance of central versus peripheral factors, especially because the development of central fatigue during prolonged maximal efforts is to a large extent influenced by peripheral factors. Furthermore, central and peripheral fatigue mutually influence each other, and exercise performance is limited by a combination of factors.

The original central fatigue hypothesis proposed that an increase in brain serotonergic activity occurring during prolonged exercise may augment lethargy and loss of drive, resulting in a reduction in motor unit recruitment, and thereby affecting the physical and mental efficiency of athletes. Since the publication of the serotonin hypothesis,[7,8] numerous theories involving accumulation or depletion of different substances in the brain have been proposed to explain central fatigue. Although the theoretical rationale for the 'serotonin-fatigue hypothesis' is clear, several seemingly well conducted studies have failed to support a significant role for serotonin in the development of fatigue. However, it is probably premature to discount serotonin entirely. It has been suggested that serotonin may play an important role in fatigue during very long duration exercise[94] and it is distinctly possible that serotonin may act in a neuromodulatory role, indirectly influencing fatigue through its action on other systems within the CNS.

As brain function appears to be dependent upon the interaction of a number of systems, it is unlikely that a single neurotransmitter system is responsible for central fatigue. It is likely that several other mechanisms are involved, with evidence supporting a role for the brain catecholamines, adenosine and NH_3. A number of subtle control factors have also been proposed to direct brain neurochemistry, and although little is known about the neurophysiological and psychological basis for central fatigue, it is likely that the interaction of cerebral metabolic, thermodynamic and hormonal responses during prolonged exercise will determine the delicate communication between the brain and the periphery. Fatigue is therefore likely to be an integrated phenomenon with complex interaction among central and peripheral factors.

Exercise capacity and performance is significantly diminished when exercise is undertaken in warm environmental conditions. The established peripher-

al mechanisms that account for the development of fatigue when exercising in temperate conditions do not appear to play a significant role, leading to the suggestion that the CNS is important. Evidence that hyperthermia has a profound effect on cerebral activity, muscle activation and perceived exertion during exercise supports this view. While it is currently unclear how an elevated body temperature contributes to the development of fatigue, it seems possible that a critical core temperature may serve as a protective mechanism preventing potential damage to the body by limiting further heat production. As evidence for a role of serotonin during exercise in the heat is limited, central catecholamines may be an important mediator of this response, and manipulation of brain dopamine and noradrenaline has been demonstrated to enhance exercise performance in the heat. Again, several neurotransmitter systems seem to be involved in controlling the functional role of PO/AH in thermoregulation during exercise.

To date, work in this area has largely relied on observations of external behaviour (exercise capacity, mood etc.) following the administration of nutritional or pharmacological interventions. While this can give an insight into the role of the CNS in the development of fatigue, we are still in the dark over the exact functional effect of brain systems. With the development of brain imaging techniques (PET, functional magnetic resonance imagery etc.), *in vivo* brain microdialysis and the measurement of arterial-venous difference across the brain we can hope to elucidate the specific neurobiological mechanisms of fatigue during exercise in the near future.

Acknowledgements

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