Ammonia metabolism, the brain and fatigue; revisiting the link

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ABSTRACT

This review addresses the ammonia fatigue theory in light of new evidence from exercise and disease studies and aims to provide a view of the role of ammonia during exercise. Hyperammonemia is a condition common to pathological liver disorders and intense or exhausting exercise. In pathology, hyperammonemia is linked to impairment of normal brain function and the onset of the neurological condition, hepatic encephalopathy. Elevated blood ammonia concentrations arise due to a diminished capacity for removal via the liver and lead to increased exposure of organs, such as the brain, to the toxic effects of ammonia. High levels of brain ammonia can lead to deleterious alterations in astrocyte morphology, cerebral energy metabolism and neurotransmission, which may in turn impact on the functioning of important signalling pathways within the neuron. Such changes are believed to contribute to the disturbances in neuropsychological function, in particular the learning, memory, and motor control deficits observed in animal models of liver disease and also patients with cirrhosis. Hyperammonemia in exercise occurs as a result of an increased production by contracting muscle, through adenosine monophosphate (AMP) deamination (the purine nucleotide cycle) and branched chain amino acid (BCAA) deamination prior to oxidation. Plasma concentrations of ammonia during exercise often achieve or exceed those measured in liver disease patients, resulting in increased cerebral uptake. In this article we propose that exercise-induced hyperammonemia may lead to concomitant disturbances in brain function, potentially through similar mechanisms underpinning pathology, which may impact on performance as fatigue or reduced function, especially during extreme exercise.

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1. Introduction: The ammonia fatigue theory

Ammonia is usually considered to be a waste product of the metabolism of amino acids and other nitrogenous compounds. For normal function of an organism it has to be removed as quickly as possible, otherwise consequences of its toxicity may be life threatening. In lower organisms, e.g. fish, ammonia removal is achieved across gills and skin. The precise mechanism is still poorly understood and may involve pH differences promoting a transmembrane gradient for gaseous diffusion of NH$_3$, or specific transporters such as the Rhesus protein family to transport ammonia (Wright and Wood, 2009). In higher organisms the products of nitrogen metabolism are voided as uric acid (birds and reptiles) or urea (mammals), although each of these different organisms may make both compounds in varying amounts, dependent on nutrition, activity and errors in purine or amine metabolism determined by genetic factors, e.g. gout. When ammonia metabolism, or its removal, is altered there are a range of symptoms that arise indicating a potentially causal role of ammonia in the observed behavioural and metabolic disturbances.

Fatigue is synonymous with a wide spectrum of familiar physiological conditions, from pathology and general health, to sport and physical exercise. Although a common phenomenon, the mechanisms involved in its development remain elusive. Numerous mechanisms and contributory factors have been implicated in the development of fatigue over the years, these include, amongst others:

- Build up of peripheral toxins/metabolic by-products/changes in peripheral environment (Ferreira and Reid, 2007; Fitts, 2008).
- Centrally mediated self regulation (Noakes et al., 2004).
- Inflammatory cytokine production (Gleeson, 2000; Robson-Ansley et al., 2004; Carmichael et al., 2006).
- Alterations in neurotransmitter metabolism (Meeusen et al., 2006).
- Periphery regulated central drive control (Amann and Dempsey, 2008a; Amann and Dempsey, 2008b).

With many more likely to be proposed as our knowledge of the physiology of fatigue evolves. For an overview of these other mechanisms we guide the reader towards the above cited literature. This review will concentrate solely on the suggested link between ammonia and fatigue.

The suggestion that ammonia accumulation has a significant role in fatigue is not new. Tashiro (1922) was the first to question a link between the production of ammonia and fatigue, after noting that there was an increased release of ammonia from isolated nerve fibres after electrical stimulation. This finding combined with the discovery of ammonia production in muscle by the breakdown of AMP during intense or stimulated contraction (Parnas, 1929; Lowenstein, 1990; Rundell et al., 1992; Hisatome et al., 1998), led many early researchers in exercise physiology to investigate this relationship further. It was soon established that there was an intensity dependent relationship between plasma ammonia concentration and exercise, with minimal change in concentration at intensities below 50–60% of VO$_2$max, but a rapid increase at intensities greater than this up to maximal exhaustion (Babij et al., 1983; Buono et al., 1984). Significant ammonia production and release was not solely restricted to intense exercise however, further investigation found that during prolonged (>1 h) submaximal exercise (50–75% VO$_2$max), ammonia could be produced in increasing amounts through the breakdown of BCAA for additional energy provision (Kasperek et al., 1985; Wagenmakers et al., 1990; van Hall et al., 1995). It was this relationship between the presence of large amounts of ammonia in muscle and blood at maximal exercise and exhaustion, that provided many researchers with evidence to suggest a potentially causal link between ammonia and fatigue processes (Mutch and Banister, 1983; Banister and Cameron, 1990; Spodaryk et al., 1990). However, as is now known from the assumptions made with lactate and fatigue, peripheral accumulation of metabolic by products does not necessarily infer a causal relationship (Philp et al., 2005).

Sixty years after the idea was initially conceived, Eric Banister and colleagues used the evidence provided by the link between exercise and muscle-derived ammonia production, with that of clinical studies where ammonia concentrations had clear clinical correlates to the central nervous system (CNS), to propose the ammonia fatigue theory in three review papers (Mutch and Banister, 1983; Banister et al., 1985; Banister and Cameron, 1990). Ammonia had long been considered a major cause of neurological disturbances in a number of pathologies, ever since it was observed that increases in blood ammonia concentration were associated with the development of stupor in portacaval shunted animals and humans (Hahn et al., 1893; Nencki et al., 1895; McDermott Jr. and Adams, 1954). Ammonia had also been implicated in the onset of convulsions in rats (Banister et al., 1976; Singh and Banister, 1981), as well as being observed to reduce contractility of muscle in vitro after exposure to high concentrations of ammonia (Heald, 1975). These findings indicated a causal role for ammonia in CNS and neuromuscular dysfunction, which led Banister and colleagues to argue that the increased production, accumulation and distribution of ammonia during exhaustive exercise, could lead to similar disturbances in function and contribute to the onset of fatigue and an inability to sustain efficient or maximal exercise performance (Mutch and Banister, 1983; Banister and Cameron, 1990). However, a major problem with this theory at the time was the lack of strong empirical evidence for a role of ammonia in fatigue, consequently it was overshadowed by other emerging theories, such as the Central Fatigue Serotonin Hypothesis (Blomstrand et al., 1988; Newsholme et al., 1992).

In recent years the main foundation of the ammonia fatigue theory, the pathological disorder hepatic encephalopathy (HE), has received increased research attention, the results from which seem to provide more supportive evidence to what was originally suggested by Banister and colleagues. Fatigue remains a
controversial topic in the literature and it may, therefore, be time to revisit the ammonia fatigue theory once more. The following review aims to provide an overview of ammonia metabolism during exercise and pathology, as well as re-evaluating the proposals of Banister and colleagues using new evidence, whilst summarising the more recent findings and future considerations for this topic.\(^1\)

2. Ammonia production in the body

Ammonia is an important metabolic end product and intermediate of several biochemical pathways in the body, its appearance in the systemic circulation stems from a number of sources (gut, muscle, kidney, brain; Olde Damink et al., 2002). Under normal physiological conditions, the majority of systemic ammonia is released from the gut or gastrointestinal (GI) tract (Summerskill and Wolpert, 1970; Romero-Gómez et al., 2009). Here nitrogenous compounds, primarily glutamine and urea, in addition to epithelial and bacterial debris, are broken down through a combination of proteases, urease containing bacterial flora and ammonia liberating enzymes such as glutaminase, of which activity in the GI tract is very high (James et al., 1998; Olde Damink et al., 2002). This bacterial and enzymatic activity releases large amounts of ammonia, which through a combination of passive diffusion and active transport mechanisms, such as the recently identified rhesus non-erythroid glycoprotein B (RhBG) and C (RhCG; Handlogten et al., 2005), are transported across the mucosal epithelium into the hepatic portal circulation (Olde Damink et al., 2009). As little as 1% of the ammonia remains in the GI tract to be excreted in the faecal matter (Summerskill and Wolpert, 1970).

The hepatic portal vein delivers ammonia to the liver, where it may be incorporated into either urea via ureagenesis within the periportal hepatocytes (hepatocytes present around the portal vein), or glutamine via the glutamine synthetase (GS) reaction within the perivenous hepatocytes (hepatocytes present around the hepatic vein) (Haussinger, 1983; Haussinger et al., 1992; Olde Damink et al., 2009). This compartmentalised system of detoxification (Fig. 1) is highly efficient, with the resting healthy adult liver able to remove all ammonia delivered by the portal circulation in a single pass, even with fluctuations in nutritional state and variability in protein content of meals (Yang et al., 2000). Once formed, urea and glutamine re-enter the circulation and through a complex system of inter-organ exchange with the kidneys and GI tract, are either excreted from the body in urine, or utilised to maintain the acid-base and nitrogen balance (for more information on these processes see Patterson et al., 1995; Hamadeh and Hoffer, 1998; Yang et al., 2000; van de Poll et al., 2004). This efficient system of detoxification and inter-organ exchange ensures that plasma ammonia concentrations are maintained within a low range of no greater than 50–100 \( \mu \text{mol} \text{l}^{-1} \) (Felipo and Butterworth, 2002). Exchange between the GI tract, liver and kidneys maintain most of ammonia homeostasis, however, other tissues and organs, such as the brain and skeletal muscle also contribute to ammonia metabolism and regulation (Olde Damink et al., 2002; Olde Damink et al., 2009). Skeletal muscle makes up approximately 40% of total body mass (Shimomura et al., 2006), and has a large potential capacity for the production, uptake and metabolism of ammonia. Some early tracer studies estimated that approximately 50% of ammonia may be metabolised in muscle to form glutamine, via the GS reaction (Lockwood et al., 1979). However, GS activity (Lund, 1970) and ammonia uptake is low in resting muscle (Eriksson et al., 1985; Bangsbo et al., 1996) and there are large differences in uptake between skeletal muscle groups in the body (Webster and Gabuzda, 1958; Ganda and Ruderman, 1976). More recently it has been estimated that, in healthy humans, ammonia uptake by skeletal muscle at rest may be close to zero, indicating that muscle may not contribute significantly to ammonia metabolism in the healthy resting adult (Olde Damink et al., 2002). Even so, muscle may become more important under conditions when other methods of removal deteriorate, as is the case for liver disease (Clemmensen et al., 2000), and when production far exceeds the capacity for removal, such as in exercise (Banister and Cameron, 1990; Bangsbo et al., 1996).

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\(^{1}\) Ammonia occurs in two forms; the protonated ammonium ion \( (\text{NH}_4^+) \) and non-protonated ammonia gas \( (\text{NH}_3) \). Where appropriate delineation is needed, the specific form will be highlighted, otherwise the word ammonia will be used to describe both the protonated and non-protonated forms combined.

![Diagram of ammonia detoxification within the liver](image-url)
Ammonia metabolism in a healthy adult at rest is a cyclical process (Fig. 2), with several input and output points. Even the most minor of alterations to any part of this process may affect ammonia homeostasis, leading to the need for changes in the system to cope with this perturbation. Liver dysfunction and exercise act to provoke a change in homeostasis either by diminishing capacity for removal or increasing production of ammonia. Impaired neurological function is well documented in liver disease (Weissenborn et al., 2005) and closely associated with diminish capacity for removal or increasing production of ammonia. Alterations in ammonia metabolism (Felipo and Butterworth, 1998) could be a cause of its effects on ammonia metabolism? Before considering this question, it is important to understand the mechanisms behind exercise induced hyperammonemia.

3. Ammonia accumulation during exercise

3.1. Adenosine monophosphate (AMP) deamination

The discovery of ammonia production by muscle was first reported by Parnas and colleagues in the late 1920s (Parnas and Mozolowski, 1927). They found a close relationship between ammonia formation in muscle, anaerobic work and adenine nucleotide degradation (Parnas, 1929). It was discovered that the reaction,

\[
\text{AMP} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{IMP} + \text{NH}_4^+ \quad (1)
\]

controlled via the enzyme AMP deaminase (AMPD), was responsible for the observed ammonia production in muscle (Schmidt, 1928; Parnas, 1929). This discovery was later identified as part of a process called the purine nucleotide cycle (PNC) which involves three interlinked reactions controlled by the enzymes AMPD, adenylosuccinate synthetase (AS) and adenylosuccinate lyase (AL).

1. \[\text{AMP} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{IMP} + \text{NH}_4^+ \quad (1')\]
2. \[\text{IMP} + \text{GTP} + \text{Aspartate}^{\text{AS}} \rightarrow \text{Adenylosuccinate}^{\text{AL}} + \text{GDP} + \text{P}_i \quad (2)\]
3. \[\text{Adenylosuccinate}^{\text{AL}} + \text{AMP} \rightarrow \text{Fumarate}^{\text{AL}} \quad (3)\]

Metabolic flux through this pathway is tightly regulated by the cell to maintain adequate levels of adenine nucleotides and TCA cycle intermediates for cell energy production (Tullson and Terjung, 1990; Hisatome et al., 1998). Under a resting physiological state, i.e. no muscle contraction, approximately 90% of the skeletal muscle AMPD is in a sarcoplasmic position and as an inactive form (Rundell et al., 1992). However, a significant change occurs as intense muscle contraction begins, when approximately 50–60% of this AMPD becomes bound to the myofibril (Rundell et al., 1992). Binding of the enzyme increases its activity causing an increased rate of degradation of AMP to IMP. This increased breakdown of AMP will affect the equilibrium of the adenylate kinase (AK) reaction creating additional ATP from ADP

\[
2\text{ADP} + \text{ATP} + \text{AMP} \rightarrow 2\text{ADP} + \text{AMP} \quad (4)
\]
to increase the cellular energy charge and maintain contraction under conditions of increasing stress (Hisatome et al., 1998).

Early investigations by Wheeler and Lowenstein suggested that several regulators of AMPD activity existed, the most potent of these were thought to be increases in ADP and reductions in cellular pH (Wheeler and Lowenstein, 1979). During intense exercise, when AMP production and deamination are high, ADP levels also increase, as utilization of ATP exceeds rephosphorylation and muscle pH decreases significantly from 7.08 to 6.60 (Sahlin et al., 1976). According to Wheeler and Lowenstein this would create ideal conditions for high activity of AMPD to enable high rates of AMP deamination. However, AMPD activity is not dependent on cellular acidosis, as was first thought, binding of AMPD to the myofilament is not significantly different during intense contraction with or without acidosis (Rundell et al., 1992). Regulation of AMPD seems to be more dependent on the isozyme present in muscle fibre rather than pH and ADP levels (Hisatome et al., 1998).

AMPD is found in a wide variety of different tissues in the body. In adult skeletal muscle the isoform AMPD1 predominates (Hisatome et al., 1998). It is believed that the properties of the AMPD1 form controls its binding to the myofibrils at the myosin heavy chain and its activity (Hisatome et al., 1998). Studies which have investigated the properties of AMPD1 in more detail have identified two alternative transcripts. The primary transcript of the AMPD1 gene is subject to alternative splicing at the 12 base miniexon; exon 2 (Mineo et al., 1990; Morisaki et al., 1993). This leads to exons either being removed producing the exon 2 deleted (E2−) transcript, or remaining producing the exon 2 (E2+) transcript (Mineo et al., 1990; Morisaki et al., 1993). The abundance of these two AMPD1 transcripts is dependent on the tissue type where they are expressed (Morisaki et al., 1993). E2+ AMPD1 is the predominant transcript in fast-twitch glycolytic fibres and is inactive (free/unbound) when ATP is bound to a regulatory site on the transcript (Hisatome et al., 1998). Therefore, at rest or during low intensity exercise when ATP resynthesis is in a steady state and the cellular energy charge is easily maintained, AMPD1 E2+ remains inactive due to the bound ATP (Hisatome et al., 1998). However, when intense muscle contraction occurs and use of ATP increases, the inhibitory effect is reduced. This allows AMPD1 to bind to the myosin heavy chain at the S2 subregion, converting AMPD1 E2+ to its active form, catalyzing deamination of AMP to IMP and ammonia, and improving energy provision through the AK reaction (Hisatome et al., 1998).

Unlike E2+, the E2− transcript of AMPD1 is believed to have a very different role. E2− is abundant in all muscle fibre types and is active when concentration of ATP is high (Hisatome et al., 1998). It is thought that this transcript, by either neural or hormonal stimulus, overcomes ATP inhibition to control flux through the PNC under stable cellular conditions, thus maintaining levels of TCA cycle intermediates for cellular energy production (Hisatome et al., 1998). This is still unclear however, and further investigation is warranted.

Residual deamination should increase IMP concentration. IMP would normally be reaminated via the adenylosuccinate synthetase (AS; Eq. (2)) and adenylosuccinate lyase (AL; Eq. (3)) reactions. However, the activity of AMPD is higher in skeletal muscle than AS, the rate limiting enzyme of the PNC. Differences in the activities of

![Fig. 2. The cyclical process of whole body ammonia metabolism at rest in a healthy individual.](Image)
these enzymes may be the reason why significant IMP accumulation is observed in muscle during exercise (Lowenstein, 1990). Some authors provide an alternative explanation for this however, as there is evidence to suggest that the enzyme AS is inhibited by a variety of substrates produced during the process of muscle contraction, AMP is a weak inhibitor and IMP a potent inhibitor of AS (Borza et al., 2003). Therefore, under conditions of high AMPD activity, which occur during intense muscle contraction, IMP levels will increase leading to inhibition of AS, preventing the reamination back to AMP, thus promoting the accumulation of IMP and ammonia in skeletal muscle (Borza et al., 2003).

Increases in muscle IMP provides the potential for a small amount of this (approximately 3% of the total adenine nucleotide pool) to be degraded further, by 5′-nucleotidase, to inosine. Inosine, in turn, may be degraded to hypoxanthine via purine nucleoside phosphorylase (Hellesen et al., 1998; Braut and Terjung, 2001). These nucleosides and bases can either be resynthesised to IMP via the purine salvage pathway (Braut and Terjung, 2001), or can cross the cell membrane and be released into the systemic circulation (Hellesen et al., 1998). Once these substrates have diffused into the circulation, it appears that they are permanently lost as adenine nucleotide precursors in muscle (Stathis et al., 1999). Therefore, with the reamination phases of the PNC less active or inactive during intense exercise, there is the potential for a reduction in the total adenine nucleotide pool, due to the degradation to hypoxanthine, thus affecting cellular energy production capacity.

The control of AMP deamination in muscle cell is complex and production of ammonia and IMP is an unavoidable consequence of maintaining muscle contraction during intense exercise. To ensure adequate energy resources for contraction, increased levels of AMP need to be deaminated and hence large amounts of ammonia will be produced. Eventually, exercise will cease due to exhaustion/fatigue, this appears to coincide with high levels of ammonia in muscle and the circulation (Spodaryk et al., 1990). This relationship between high levels of ammonia and the onset of fatigue indicates that ammonia may contribute to fatigue.

3.2. Muscle ammonia release

The proportions of ammonia in solution, as gaseous (NH₃) or ionic (NH₄⁺) form, are dependent on pH. The quantities of each may be calculated using the Henderson-Hasselbalch equation:

\[
\log 10\left(\frac{[\text{NH}_3]}{[\text{NH}_4^+]}\right) = p\text{K}_a - p\text{H}
\]  

(Marcaggi and Coles, 2001)

The \(pK_a\) of ammonia is reported to be between 9.01 and 9.25 (Bromberg et al., 1960; Katz et al., 1986; Marcaggi and Coles, 2001), and the pH range of exercising muscle lies between 7.1 and 6.6 (Sahlin et al., 1976). Using Eq. (5) approximately 99% of all ammonia inside skeletal muscle at rest or during exercise will be in the form of ammonium ions (NH₄⁺). Without a specific transport process or ion channel, ammonia release will be limited, due to poor permeability of the lipid in cell membranes to ions. The amount of ammonia released from contracting muscle during exercise has been estimated within the range of 10–25% of that produced in the muscle (Katz et al., 1986; Graham et al., 1990). Indicating that a large proportion (up to 90%) may be retained until exercise is complete, after which it may be gradually released and metabolised during the recovery phase (Graham et al., 1990).

Ammonium ion specific transporter mechanisms RhBG and RhCG have been identified in some tissues and organs, such as in the kidneys (Quentin et al., 2003), gut (Handlogten et al., 2005) and neurons (Hillmann et al., 2008). However, ammonium specific transport mechanisms remain to be identified in skeletal muscle (Liu et al., 2000; Liu et al., 2001). Therefore, NH₄⁺ ion transport is likely to be low and may be a limiting factor in the release of ammonia from exercising muscle. NH₄⁺ may substitute for K⁺ due to their similar size and charge (Ott and Larsen, 2004), and may therefore exit cells via K⁺ channels or transporters, routes which have been identified in astrocytes (Nagaraja and Brookes, 1998) and intestinal membranes (Hall et al., 1992). These processes are abundant in skeletal muscle and may act to remove NH₄⁺ from muscle, although the high intramuscular concentration of potassium will compete for the same processes (Payne et al., 1995).

Resting intramuscular potassium concentrations have been reported as approximately 165 mM, and can decline to approximately 130 mM after intense exercise (McKenna et al., 2008). This is far greater than intramuscular ammonia concentrations at rest or after exercise (approximately 1 mM maximum; Katz et al., 1986).

Retention of ammonia in skeletal muscle may be beneficial to the body. Low permeability will reduce the rate of release of muscle ammonia and hence reduce the concentrations reached in the blood during exercise. If ammonia were to be released more quickly then blood concentrations would be predicted to rise to mM levels after very intense or exhausting exercise, which may then lead to other more serious consequences, as will be highlighted later in this review.

3.3. Exercise intensity dependent relationship to blood ammonia

Production of ammonia in muscle from the PNC suggests that ammonia production should be dependent on the intensity of the exercise. Studies using rat and human subjects found this to be the case with more ammonia being produced at high intensities (70–110% VO₂max; Dudley et al., 1983; Broberg and Sahlin, 1989), in 1:1 stoichiometry with IMP (Meyer and Terjung, 1979; Katz et al., 1986). Whereas, at intensities below 50% VO₂max, little to no accumulation is observed (Babij et al., 1983; Buono et al., 1984; Katz et al., 1986). This relationship between exercise intensity and muscle ammonia production is curvilinear. A relatively small amount of ammonia production is observed up to approximately 50–60% VO₂max, above this ‘threshold’ level blood ammonia is observed to accumulate rapidly with increasing exercise intensity (Babij et al., 1983; Buono et al., 1984). Because of this relationship, researchers believed for many years that AMP deamination was the only significant pathway through which ammonia was formed during exercise. This interpretation was most likely due to the restricted methodologies used to investigate this phenomenon, such as short duration intense exercise (Dudley et al., 1983; Broberg and Sahlin, 1989) or incremental exercise to exhaustion (Buono et al., 1984). Many overlooked other potential sources of ammonia production in muscle, i.e. branched chain amino acid (BCAA) deamination (Graham et al., 1987, 1995a). This may have been due to the general consensus that amino acid metabolism contributes little to energy production during exercise and was deemed less significant than AMP deamination (Graham et al., 1995a). However, this is not the case as anything up to 10% of total energy produced during exercise of a prolonged duration can come from amino acid oxidation and utilisation of the BCAA pathways (Brookes, 1987).

3.4. Branched chain amino acid (BCAA) deamination

The branched chain amino acids (BCAA): leucine, isoleucine and valine, make up approximately 40% of dietary essential amino acids (EAA) and play vital roles in the structures of globular and membranous proteins due to their strong hydrophobicity (Brosnan and Brosnan, 2006). Unlike other EAA, BCAA largely escape hepatic
catabolism and are metabolised extra-hepatically, with the majority of this taking place in the mitochondria of skeletal muscle (Brosnan and Brosnan, 2006). Through this process (Fig. 3), the BCAAs are broken down via two reactions; branched chain aminotransferase (BCAT) and branched chain alpha keto acid dehydrogenase (BCKDH), to form coenzyme A compounds which can be utilised in the TCA cycle for oxidative energy production (Shimomura et al., 2004, 2006). At the primary BCAT reaction, the amino group from the BCAA is utilised to form glutamate from 2-oxoglutarate, after which this glutamate can then either form glutamine via GS, or alanine through combination with pyruvate. In some cases glutamate may react with the co-factor NAD⁺ via the glutamate dehydrogenase (GDH) reaction leading to the formation of ammonia, identifying that AMPD is not the only pathway in muscle by which ammonia generation may take place (Wagenmakers et al., 1990). However, because of the stoichiometry of ammonia accumulation to AMP deamination (Meyer and Terjung, 1990; Wagenmakers et al., 1990), ammonia production via BCAA deamination was thought minimal. This idea remained until the activation of the rate limiting step of BCAA catabolism was found to be linked to exercise (Kasperek et al., 1985).

The rate limiting step is controlled by the BCKDH enzyme complex (Fig. 3). The activity of this enzyme complex is regulated by a phosphorylation-dephosphorylation cycle (Shimomura et al., 2004). Conversion to its phosphorylated, inactive, form is controlled via BCKDH phosphorylation (Shimomura et al., 2004). At rest, the complex is largely phosphorylated and inactive (Shimomura et al., 1995), however, during exercise the activity of the BCKDH complex may increase up to 10-fold in rat muscle (Kasperek et al., 1985), and 4-fold in human muscle (Wagenmakers et al., 1989). As exercise progresses, there is an increased breakdown of BCAA in muscle via BCAT (Wagenmakers et al., 1990; Bowtell et al., 1998). This increases the levels of branched chain keto acids (BCKA) in muscle, which have been found to be potent inhibitors of BCKDH kinase activity (Paxton and Harris, 1984). In addition, the amount of the bound (active) form of BCKDH kinase has been found to decrease in muscle during exercise (Xu et al., 2001). Both actions will assist in increasing the activation of the BCKDH enzyme complex, which will in turn lead to an increase in flux through the BCAT pathway, thereby increasing the substrate availability for ammonia production via GDH (Fig. 3). With sufficient pyruvate available the amino group provided by the glutamate will be preferentially incorporated into alanine, due to the near equilibrium state of the alanine aminotransferase reaction (Wagenmakers et al., 1990; Wagenmakers, 1998). The majority of the ammonia produced via this pathway is only likely to occur when the availability of pyruvate is reduced, as glycogen stores are depleted (Wagenmakers et al., 1990). Supporting evidence can be observed in individuals with myophosphorylase deficiency (McArdle’s Disease), who are unable to utilise glycogen as an energy source. During exercise they need alternative sources of energy and BCAA catabolism plays a part in this (Wagenmakers et al., 1990). In such individuals ammonia accumulation is much greater than healthy controls (leg muscle ammonia efflux; 402 μmol min⁻¹ in McArdle’s Disease patients at 70%Wmax, 46 μmol min⁻¹ in healthy individuals at equivalent intensity; Wagenmakers et al., 1990). These findings therefore suggest that ammonia accumulation via this pathway is likely to be most active in states of glycogen depletion, such as towards the end of endurance exercise when pyruvate levels may be reduced and BCAA metabolism is increased (Wagenmakers, 1998).

Ammonia, however, has been shown to be produced early on in submaximal (60–65% of max power output) endurance exercise even in the absence of AMP deamination (van Hall et al., 1995), suggesting that ammonia may be produced by BCAA catabolism, independent of glycogen availability (van Hall et al., 1995). This is supported further by results showing that ammonia efflux from muscle during such exercise was similar in both normal and glycogen depleted states (van Hall et al., 1995). These findings indicate that BCAA catabolism and the utilisation of AA in muscle may be vital to the energy production pathways during exercise even in the presence of adequate fuel and energy substrates. However, others have found that the ingestion of carbohydrates, to increase glycogen stores, prior to prolonged submaximal exercise leads to an attenuation of ammonia production (Wagenmakers et al., 1990).
et al., 1991; Snow et al., 2000) and that the activity of the BCKDH complex is greater in glycolgen depleted muscle (van Hall et al., 1996).

The consensus view is that the production of ammonia during exercise occurs via a combination of both AMP deamination and BCAA metabolism, which are activated in an intensity and duration dependent manner. The production of ammonia can lead to significantly elevated systemic ammonia levels being observed of anything between 90 and >200 μmol l⁻¹ (Hellsten et al., 1999; Dalsgaard et al., 2004; Nybo et al., 2005; Mohr et al., 2006). This is a 3–10 fold increase on levels commonly observed in healthy individuals at rest (20–80 μmol l⁻¹: van Hall et al., 1995; Bangsbo et al., 1996). The only other known conditions in which such acute increases in ammonia concentration are observed, are in the presence of certain pathological complications, where such high ammonia levels are believed to lead to serious deteriorations in health and well being.

4. Pathophysiological effects of ammonia

The main focus and rationale for the ammonia fatigue theory stems from the psychological derangements observed in pathological conditions where hyperammonemia is believed to be a major precipitating factor (Felipo and Butterworth, 2002);

- Liver fibrosis/cirrhosis due to chronic liver disease.
- Liver failure due to an acute insult of the liver tissues.
- In-born urea enzyme deficiencies, such as ornithine transcarbamoylase (OTC) deficiency.

There is a long history of investigation into the role of ammonia in the pathophysiology of such conditions. From the primary identification of developing stupor in portacaval shunted dogs, and later in humans, fed high protein diets to induce increases in blood ammonia levels (Hahn et al., 1893; Nencki el al., 1895; McDermott Jr. and Adams, 1954), to the present day in vitro studies investigating the mechanistic properties of ammonia exposure in isolated neuronal and astroglial cells (Chan and Butterworth, 2003; Caiul et al., 2009a). Such continued interest has enhanced our knowledge and understanding of some of the many mechanisms and targets involved in ammonia toxicity.

There are two main processes in pathology which contribute to the increased concentration of ammonia in the blood (Old Damink et al., 2009):

- Portacaval shunting; whereby anastomoses (either artificial or congenital) allow portal blood to flow directly into the vena cava, by-passing the liver.
- Impaired liver function, either due to an in-born urea cycle enzyme deficiency or dysfunction of the hepatocellular mechanisms through the onset of disease or acute insult.

The observation that the hyperammonemic state was often accompanied by neuropsychological dysfunction in the form of Hepatic Encephalopathy (HE), has led many researchers and clinicians to conclude that ammonia plays a major pathogenic role in the development of such dysfunction (Katayama, 2004).

4.1. Pathogenic mechanisms of hyperammonemia

Brain tissue, unlike the liver, has no urea cycle, and blood-borne ammonia taken up across the blood brain barrier (BBB) is detoxified by a different process, incorporation into glutamine through the GS reaction, in the same way as in the skeletal muscle and perivenous hepatocytes (Felipo and Butterworth, 2002; Butterworth, 2003). In the brain, this process takes place primarily in the astrocyte cells, as these cells are the first point of contact for any substances entering the brain tissues from the blood (Wang and Bordey, 2008). Astrocyte cells provide protection to the neuron and perform important metabolic functions to assist in the process of neurotransmission and neuronal energy metabolism, of which ammonia forms a vital part (Wang and Bordey, 2008). Some of these functions are illustrated in Fig. 4A and B. Ammonia, through its incorporation into glutamine, plays an important role in the glutamatergic neurotransmitter system, assisting in affecting, regulating and maintaining levels of the excitatory neurotransmitter glutamate (Felipo and Butterworth, 2002). Glutamate is an important neurotransmitter and also acts as a precursor for production of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), through the action of the enzyme glutamic acid decarboxylase (Bak et al., 2006). In addition, a small proportion of the ammonia may be involved in the maintenance of neuronal energy metabolism, through an alanine/ammonium shuttle (Coles et al., 2008). This recently proposed mechanism suggests that ammonia can be utilised along with pyruvate, provided via astrocyte glucose metabolism, to create alanine through the alanine aminotransferase (AAT) reaction (Tsacopoulos et al., 1997; Coles et al., 2008). Alanine may then be shunted from the astrocyte to the neuron where it is broken down releasing pyruvate for the oxidative metabolism of neuronal mitochondria (Coles et al., 2008). The ammonia which is also released from the breakdown of alanine in the neuron is then returned to the astrocyte where it can be utilised once again (Coles et al., 2008; Fig. 4B). Ammonia, therefore, is implicated in both excitatory and inhibitory pathways through its role in the synthesis of glutamate and GABA, whilst also being important in the maintenance of neuronal energy metabolism through the alanine/ammonium shuttle. Consequently its presence should not always be considered as having a pathological effect.

As with many other systems in the body, the glutamatergic, GABA-ergic and neuronal metabolic systems are closely regulated (Bak et al., 2006; Coles et al., 2008). Under normal conditions these systems function efficiently to maintain adequate neuronal processing (Coles et al., 2008; Caiul et al., 2009a). A major imbalance may arise when blood ammonia levels start to increase, due to a failure of the main detoxification systems, as is the case in liver pathology, or an increase in production levels such as during exercise. Initially any increases may be offset by increases in the production of alanine (Tsacopoulos et al., 1997) and glutamine (Tanigami et al., 2005; Jayakumar et al., 2006) which may act as metabolic sinks within the astrocyte. Astrocytes, however, may only have a limited capacity and may reach saturation levels quickly (Cooper et al., 1985). In addition, the beneficial role of these compounds as sinks for ammonia may be questionable, as increases in glutamine concentrations in the brain have been found to have significant toxic effects (Rama Rao et al., 2003; Jayakumar et al., 2004). Eventually, ammonia concentrations will start to increase in the brain, which may lead to impairment at several sites in the cellular and subcellular processes of the astrocytes and neurons (Felipo and Butterworth, 2002; Caiul et al., 2009a). This thereby indicates that ammonia may influence brain function in positive ways at low concentration, by providing necessary substrate for neuronal metabolism and neurotransmission, and negative ways at high concentration by impairing normal cellular function.

The flux of water and other molecules from the blood into the brain is regulated by the BBB (Hawkins et al., 2006) and the movement of ammonia across cellular membranes is limited by chemical form and/or the presence of appropriate transport mechanisms (Ott and Larsen, 2004). Increased levels of ammonia in the blood lead to an increase in cerebral uptake across the BBB (Lockwood et al., 1991; Dalsgaard et al., 2004; Nybo et al., 2005;
Fig. 4. (A) Cerebral ammonia metabolism and glutamatergic neurotransmission. Ammonia can be taken up into the brain either; (a) as a substitute for K⁺, (b) using an ammonium specific transporter (RhGB or RhGC), or (c) via diffusion. It is then consumed within a cycle regulating the production of the excitatory neurotransmitter glutamate. The release of glutamate from the presynaptic neuron activates receptors on the postsynaptic membrane, which depending on the receptor subtype will activate different signalling pathways within the postsynaptic neuron. These pathways help to regulate important cellular functions. Two of these pathways are illustrated; (1) NMDA-NO-cGMP pathway. (2) mGluR G-protein coupled pathway. To prevent excess stimulation of the pathways, once activated the glutamate is removed via transporter proteins in the astrocyte membrane, and the whole cycle is initiated once more. (B) Neuronal energy metabolism and the ammonium/alanine shuttle. Ammonia may also be utilised to assist in the maintenance of neuronal energy metabolism. Ammonia within the astrocyte can form alanine with pyruvate via the AAT reaction. This alanine is then shuttled from the astrocyte to the neuron where it is broken down to release pyruvate which can be used by mitochondria for oxidative energy metabolism. The ammonia released can then be shuttled back to the astrocyte and utilised once more. AAT, alanine aminotransferase; Ala, alanine; cGMP, cyclic guanosine monophosphate; Cm, calmodulin; Gln, glutamine; Glu, glutamate; Gluc, glucose; Gluc-6-P, glucose-6-phosphate; GS, glutamine synthetase; GTP, guanosine triphosphate; mGluR, metabotropic glutamate receptor; NH₃, ammonia gaseous form; NH₄⁺, ammonium ion; NMDA, N-methyl-D-aspartate receptor; nNOS, neuronal nitric oxide synthetase; NO, Nitric Oxide; PAG, phosphate activated glutaminase; PDE, phosphodiesterase; PKC, protein kinase C; PKG, protein kinase G; PLC, phospholipase C; Pyr, pyruvate; sGC, soluble guanylate cyclase.
Keiding et al., 2006b). This seems to be the case in healthy and diseased states (Lockwood et al., 1991; Dalsgaard et al., 2004; Nybo et al., 2005; Keiding et al., 2006b). Healthy individuals performing exercise which induces significant hyperammonemia, show a shift from a net balance of ammonia flux at rest, to a significant positive cerebral uptake during exercise (Nybo et al., 2005). Similarly, in cirrhosis, researchers have reported an increased flux of ammonia into the brain of individuals with hyperammonemia (Keiding et al., 2006b).

In vitro and animal models of liver disease and hyperammonemia have identified a wide range of perturbations to the normal function of the brain which may be caused either directly or indirectly by increased ammonia concentrations, such as;

- Increased astrocyte glutamine accumulation leading to cellular oedema and astrocyte morphological and functional changes (Haussinger et al., 2000; Haussinger and Schliess, 2008).
- Induction of the mitochondrial permeability transition (MPT), reactive oxygen species production and the impairment of mitochondrial function (Albrecht and Norenberg, 2006; Norenberg and Rama Rao, 2007).

Of particular interest for the purposes of this review are the actions of ammonia on cerebral glutamate handling and glutamatergic neurotransmission (Rose, 2006; Cauli et al., 2009a). For further information on the other pathogenic consequences of ammonia toxicity we refer the reader to the above reviews.

4.1.1. Glutamatergic neurotransmission

Glutamate can interact with two types of membrane receptors on the postsynaptic neuron when released into the synaptic cleft (Monfort et al., 2005b):

- Ionotropic receptors: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and Kainate
- Metabotropic receptors: metabotropic glutamate receptor (mGluR)

These receptors once activated initiate a cascade of cellular events which, dependent on the type of receptor, will assist in regulating normal functioning within the brain. The controlled release of glutamate by neuron accompanied by the removal and metabolism of extracellular glutamate by the surrounding astrocyte, maintains neurotransmission under normal conditions. However, ammonia has been shown to significantly interfere with the processes of re-uptake when in high concentration within the brain (Rose, 2006; Cauli et al., 2009a). Of the different receptor subtypes, the effects of ammonia on NMDA receptor functioning has been the most widely studied to date (Rodrigo et al., 2009).

When glutamate activates NMDA receptors there is an influx of calcium ions into the postsynaptic neuron. Calcium then binds to calmodulin and activates the calcium dependent enzyme nitric oxide synthetase (NOS) causing an increase in nitric oxide (NO) production (Madhusoodanan and Murad, 2007). NO is an important intracellular messenger which at low concentrations activates soluble guanylate cyclase (sGC), initiating the conversion of GTP to cGMP (Fedele and Raiteri, 1999; Madhusoodanan and Murad, 2007). cGMP, like NO, is also an important intracellular signal, which, depending on the downstream cascade it stimulates, can have a number of different functional roles (Madhusoodanan and Murad, 2007). Therefore, control and tight regulation of its production and the pathways controlling its production within the neuron is essential for normal cellular function (Madhusoodanan and Murad, 2007). For example, alterations in the NMDA receptor-NO-cGMP pathway are evident in a number of pathological disease states, such as Alzheimer’s and Parkinson’s Disease (Madhusoodanan and Murad, 2007). One method of regulating this pathway is via degradation of cGMP, once its downstream target has been activated, via cGMP-degrading phosphodiesterases (PDE), which are regulated by protein kinase G (PKG), and also via the controlled release of cGMP into the extracellular space (Pepicelli et al., 2004).

Extracellular release of cGMP is a useful regulatory mechanism, as it allows for in vivo monitoring of intracellular function of this pathway using microdialysis, as changes in extracellular cGMP should represent corresponding changes in the function of the NO-cGMP pathway intracellularly (Pepicelli et al., 2004).

Ammonia affects NMDA receptors in a concentration dependent manner. In vitro and animal models of acute ammonia intoxication representative of acute liver conditions, where brain and cellular ammonia concentrations can often exceed 1 mM, have been found to lead to overstimulation of NMDA receptors and the NO-cGMP pathway, inducing increased reactive oxygen species (ROS) production in the brain (Kosenko et al., 1999; Hermenegildo et al., 2000). This overstimulation may be caused by a combination of direct stimulation by ammonia (Fan and Szerb, 1993; Hermenegildo et al., 2000), and ammonia induced disruption to astrocyte glutamate handling, leading to increased extracellular glutamate concentrations and glutamate excitotoxicity (Kimelberg et al., 1990; Bender and Norenberg, 1996; Michalak et al., 1996; Hilgier et al., 2000; Zhou and Norenberg, 1999; Chan et al., 2000; Rose et al., 2005). This increased ROS production initiates a self-amplifying chain of events which may in turn impair mitochondrial function, energy production and eventually lead to coma or death (Kosenko et al., 1999, 2003, 2007; Hermenegildo et al., 2000).

In contrast, the more moderate levels of hyperammonemia associated with chronic liver conditions, seem to have a rather different affect on the NMDA receptor, impairing it at levels beyond receptor activation (Hermenegildo et al., 1998).

Cultured neurons exposed to different moderate ammonia doses (0.01–0.1 mM) and durations of 15 min, 24 h and 6 days, showed a reduced formation of cGMP compared to controls when NMDA receptors were stimulated by glutamate (Hermenegildo et al., 1998). The effect on cGMP production was time and ammonia concentration dependent, therefore the greater the length of ammonia exposure, the greater impairment of the NMDA receptor-NO-cGMP pathway. However, only 15 min exposure was needed to produce a significant decrease in cGMP production (Hermenegildo et al., 1998).

In an attempt to assess where ammonia has its effect, the NO generating agent, S-nitroso-N-acetyl-penicillamine (SNAP), was added to the culture medium. If ammonia influenced the pathway before NO-generation, then it should be possible to reverse the reduction of cGMP via SNAP. However, cGMP formation after addition of SNAP was still significantly reduced indicating that ammonia affects the events after NO generation, possibly at sGC activity (Hermenegildo et al., 1998; Monfort et al., 2001). However, there is some evidence that may indicate effects of ammonia on earlier stages of this signalling pathway. Recent evidence points to a reduction in neuronal NOS (nNOS) activity in brain slices of hyperammonemic rats, by increased phosphorylation of nNOS, potentially implicating interference by ammonia prior to NO generation (El-Milli et al., 2008).

These in vitro findings were supported by further in vivo work. Hermenegildo et al. (1998) used brain microdialysis to stimulate the NMDA receptors in the rat cerebellum through the administration of NMDA. cGMP levels were significantly reduced by up to 60% in hyperammonemic rats, compared to control rats. Basal activity of cGMP, prior to stimulation by NMDA, was also found to be significantly reduced by up to 54% in the hyperammonemic rats (Hermenegildo et al., 1998). It is this effect of ammonia on the NMDA receptor-NO-cGMP pathway, which is believed to contribute to some of the cognitive and neurological disturbances often
observed in those with liver disease and other hyperammonemia related disorders.

4.1.1.1. Impairments in learning and memory. There is mounting evidence to suggest that regulation of the NMDA receptor-NO-cGMP pathway is essential for control of learning and memory and that disruption to this pathway may lead to impairments in cognitive function (Bernabeu et al., 1996, 1997; Monfort et al., 2009). Blocking the activation of the NMDA receptor-NO-cGMP pathway using a NMDA antagonist, dizocilpine, results in a dose dependent impairment in performance of a Y maze conditional discrimination learning task in mice (Yamada et al., 1996). This impairment was associated with a reduction in cGMP levels in the cerebellum of the mice and after administration of Br-cGMP, a cGMP analogue, the dizocilpine induced impairment was reversed (Yamada et al., 1996). The effect on the NMDA receptor-NO-cGMP pathway, by dizocilpine, was similar to that reported by Hermenegildo et al. (1998) in hyperammonemic rats. cGMP was reduced by approximately 50% in the cerebellum compared to controls (Yamada et al., 1996). Therefore, it may be expected that memory and learning would be disrupted in a similar manner in these animal models of hyperammonemia. Erceg et al. (2005) found a significant impairment in the same Y maze task in rats with chronic hyperammonemia, which was accompanied by a significant reduction in extracellular cGMP levels in the cerebellum. When these levels of cerebellar cGMP in the hyperammonemic rats were artificially manipulated via the administration of cGMP or zaprinast (a pharmacological agent which prevents cGMP degradation via inhibition of its PDE), cGMP levels significantly increased and this was paralleled by significant improvements in Y maze task learning ability (Erceg et al., 2005). In contrast increases in cGMP via zaprinast or cGMP administration in control animals resulted in a decreased learning ability (Erceg et al., 2005). These results imply that under normal conditions the functioning of the NMDA receptor-NO-cGMP pathway in the cerebellum is close to its optimum. Therefore, any perturbation to these normal conditions, such as that provided by ammonia, will impact on the functioning of this pathway, contributing to associated impairments in learning and memory.

Further evidence for the importance of regulation of the NMDA receptor-NO-cGMP pathway in the brain for optimising learning and memory can be observed through its involvement in hippocampal long-term potentiation (LTP). LTP is believed to represent the neurophysiological mechanisms underlying increased synaptic efficiency and plasticity associated with long term memory formation and learning (Lynch, 2004). It has been established that LTP in the CA1 region of the hippocampus is dependent on NMDA receptor activation, and that proper induction and maintenance of the LTP in this area relies upon sequential activation of the NMDA receptor-NO-cGMP pathway (Monfort et al., 2002). Inhibition of this pathway at various stages from the activation of sGC, to the degradation of cGMP by PDE, leads to an impaired tetanus induced LTP in rat hippocampal slices (Monfort et al., 2002). Since ammonia can significantly disrupt this pathway, this is a potential route by which hyperammonemia could impair LTP and may contribute to deficits in learning and memory in models of hyperammonemia and liver disease (Aguilar et al., 2000; Mendez et al., 2009).

Hippocampal slices from hyperammonemic rats show impaired induction of LTP, via tetanic stimulation, when compared to controls, with the magnitude of the LTP reduced by almost half (Muñoz et al., 2000). In healthy controls in order to induce and maintain hippocampal LTP there must be sequential activation of the NMDA receptor-NO-cGMP pathway, which first initiates a rapid rise in cGMP, followed immediately by a sustained decline to below basal levels (Monfort et al., 2002). Hippocampal slices treated acutely with ammonia (1 mM), however, do not show this sustained decline in cGMP levels and levels remain elevated (Monfort et al., 2004). This demonstrates that hyperammonemia may impair LTP induction by interfering with the processes controlling regulation of cGMP content. It seems evident from further investigation that ammonia disrupts the efficient degradation of cGMP by preventing the activation of the cGMP degrading PDE through an inhibition of cGMP PKG (Monfort et al., 2004). This impairment is not restricted to in vitro conditions, a study using rat models of chronic hyperammonemia showed similar, yet less severe impairment of hippocampal LTP (difference in severity likely due to differences in tissue ammonia concentrations; 1 mM in vitro; 0.1 mM in vivo), again associated with disruption to the control of cGMP levels through alterations in PKG and PDE activities (Monfort et al., 2005a).

This disruption to the control of cGMP levels will likely impact on aspects of learning and memory associated with the hippocampus, in particular spatial learning, which has been observed to be reduced after pharmacological blockade of hippocampal LTP in rats (Morris, 1989). Furthermore, this type of learning was also found to be significantly disrupted in rat models of chronic liver disease, and as with chronic hyperammonemia, LTP was impaired (Monfort et al., 2007). However, this impairment was greater than that observed in the rat models of hyperammonemia alone, suggesting contribution from other precipitating factors to LTP impairment in chronic liver disease additive to ammonia (Monfort et al., 2007).

To summarise, in vitro and in animal models, moderate levels of ammonia can directly affect the molecular mechanisms believed to be involved in forms of learning and memory. These effects of ammonia seem to be brain area specific. In the cerebellum for example, evidence points towards a disruption at the level of cGMP synthesis arising from either NO inhibition (El-Milli et al., 2008), or a reduced activation of sGC (Hermenegildo et al., 1998; Monfort et al., 2001), which leads to reduced cGMP synthesis. This reduction in cGMP synthesis in turn, impacts on aspects of learning associated with cerebellar function, such as conditional discrimination learning (Yamada et al., 1996; Erceg et al., 2005). In contrast, in the hippocampus, ammonia seems to prevent the efficient degradation of cGMP, via inhibition of PKG activated PDEs (Monfort et al., 2004, 2005a), impairing both the induction and maintenance of hippocampal LTP. Consequently, hippocampal associated spatial learning abilities are disrupted (Morris, 1989; Monfort et al., 2007). Taken together these actions are likely to contribute to the observed overall learning deficits in animal models of liver disease and hyperammonemia (Aguilar et al., 2000; Mendez et al., 2009).

Similar disruption at a molecular level in humans as a result of liver disease and associated pathological hyperammonemia has not been well researched. In one study, however, autopsied brain slices from patients with cirrhosis have shown disruption in the activation of sGC compared to that of healthy brain slices, with increases in sGC activity in the frontal cortex and reductions in activity in the cerebellum (Corbalán et al., 2002). Similar regional alterations in sGC activity can be observed in rat models of liver disease (Monfort et al., 2001; Corbalán et al., 2002), and in cultured neurons exposed to ammonia levels equivalent to chronic hyperammonemia animal models (Rodrigo et al., 2005). This identifies that similar changes in the brain occur in both rat models of chronic liver disease and patients with cirrhosis. Consequently, the mechanisms constituting cognitive dysfunction in these rat models may also be present in patients with cirrhosis.

4.1.1.2. Disturbances in motor activity. Disruptions to glutamatergic neurotransmission by ammonia are not purely limited to the molecular mechanisms proposed to be involved in learning and memory. Their impact may also extend to the neural circuitry
regulating motor output. Normal motor control requires functional connectivity between a number of cerebral areas. However, it is well established that neural projections provided via the basal ganglia are highly involved in the regulation of motor output (Hauber, 1998). Neuroanatomical studies have identified that efferent projections from the nucleus accumbens (NA) innervate the prefrontal cortex (PFCx) via the ventral pallidum (VP) and thalamic nuclei (Mogenson et al., 1983; Churchill et al., 1996a, 1996b; Churchill and Kalivas, 1999). Artificial activation at the various junctions of this circuit have found an increase in motor activity or excitatory responses in the PFCx (Pirot et al., 1995; Attarian and Amalric, 1997; Meeker et al., 1998; Churchill and Kalivas, 1999). It seems evident that activation of this circuitry is dependent on the release of dopamine in the NA after activation of the metabotropic mGluR group 1 glutamate receptors (Attarian and Amalric, 1997). A number of studies have demonstrated that NA activated locomotion in rats is highly dependent on dopamine release, depletion of dopamine and the action of dopamine receptor inhibitors attenuate mGluR activated locomotion (Attarian and Amalric, 1997; Meeker et al., 1998). Dopamine receptors are known to facilitate the release of GABA from GABAergic terminals (Aceves et al., 1995), which project densely from the NA to the VP (Jones and Mogenson, 1980). The VP provides efferents extending via the medio-dorsal thalamus (MDT) to the PFCx (Churchill and Kalivas, 1999), which are again GABAergic in nature (Churchill et al., 1996a, 1996b; Churchill and Kalivas, 1999). The PFCx, in turn, provides dense projections to a number of pre-motor areas which allows this circuit direct access to the areas implicated in movement selection and initiation, and a direct link to the cortico-spinal tract (Lu et al., 1994). Although the neural interactions within these areas are numerous and highly complex in vivo, the proposed control provided by this circuitry has been simplified for the purposes of this review and is summarised in Fig. 5A.

To regulate this stimulatory pathway, neural projection from the substantia nigra pars reticulata (SNr), provides inhibitory input to the prefrontal cortex via the ventral medial thalamus (VMT) (Kemel et al., 1988; Miyamoto and Jinnai, 1994; Timmerman and Westerink, 1997). In addition to this, a direct neural connection between the NA and the SNr, is believed to allow the NA to, when stimulated, regulate the inhibitory input from the SNr to the PFCx (Montaron et al., 1996). The complex interaction of these pathways extending from the basal ganglia to the PFCx assist in maintaining appropriate levels of motor output. It is these pathways which have recently been identified as those which may be compromised under conditions of hyperammonemia and liver disease.

Using rats treated with a portacaval shunt (PCS), to produce a model of chronic liver disease with HE, it was noted that there was a significant decrease in locomotor activity observed in these rat models compared to the control rats (Cauli et al., 2006). This hypolocomotion was found to be significantly correlated to levels of extracellular glutamate in the SNr, suggesting that excess stimulation of the SNr inhibitory pathway may be responsible for decreasing locomotor activity (Cauli et al., 2006). To assess this, the PCS rats were administered a mGluR antagonist, CPCCOEt, which blocked mGluR activation in the SNr. Administration of the antagonist led to an 85% increase in motor activity in the PCS rats, but no effect in the controls (Cauli et al., 2006). These results suggest that biochemical changes which accompany liver disease may directly impair gross motor activity via disruption of the neural pathway extending from the SNr. Because this disruption is associated with an increased level of extracellular glutamate, it may implicate ammonia as a causal factor in the disruption to locomotor activity, due to its effects on astrocyte glutamate handling (Rose, 2006).

Other disruption to the neuronal circuits implicated in motor activity, have recently been identified by the same research group. Using the same model of liver disease, the ‘normal’ excitatory neuronal circuit regulating motor activity extending from the basal ganglia to the PFCx was found to be significantly disrupted in PCS rats (Cauli et al., 2007a). In control rats, stimulation of mGluR receptors in the NA using the mGluR1 agonist (5)-3,5-dihydroxyphenylglycine (DHPG), led to an increase in locomotion and coincided with the activation of the ‘normal’ neuronal circuit (Cauli et al., 2007a). In PCS rats however, activation of this circuit was absent, due to a blocking of dopamine release. Even so, there was still a significant stimulatory increase in locomotion in these rats (Cauli et al., 2007a). It was subsequently identified that locomotion in the PCS rats was achieved via an ‘alternative’ pathway which involves activation of the neural projections between the NA and SNr (Fig. 5B), providing an excitatory input to the PFCx, via the SNr and VMT, in addition to its inhibitory input (Cauli et al., 2007a). Locomotion via stimulation of this ‘alternative’ NA → SNr → VMT → PFCx pathway in PCS rats was significantly greater than that stimulated via the ‘normal’ pathway in controls.

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**Fig. 5.** Summary of the neural circuitry involved in the regulation of motor activity extending from the basal ganglia. Approximate anatomical positions of the regions involved are illustrated using the outline of a sagittal section of a rat brain. (A) Pathways involved in motor regulation in the brain of a healthy rat. Excitatory pathway extending from the nucleus accumbens (solid line), inhibitory pathway extending from the substantia nigra pars reticulata (dashed line). Neural interconnection between nucleus accumbens and substantia nigra pars reticulata (dotted line). Inhibitory inputs to the prefrontal cortex (dotted line). (B) Changes in pathway activation in PCS rats, as observed by Cauli et al. (2007a). The inactive ‘normal’ excitatory pathway (dotted line), the active ‘alternative’ excitatory pathway extending via the substantia nigra pars reticulata (solid line), NA, nucleus accumbens; MDT, medio-dorsal thalamus; PFCx, prefrontal cortex SNr, substantia nigra pars reticulata; VMT, ventro-medial thalamus; VP, ventral Pallidum.
This finding suggests that in PCS rats there may be an associative hypersensitivity, contributing to the alterations in normal motor function (Cauli et al., 2007a). Because ammonia is believed to play a major role in liver disease associated dysfunction, this same research group examined the effects of hyperammonemia in the absence of liver disease, to assess whether it was the cause of this dysfunction to locomotion was significantly increased and was associated with a reduction in hyperammonemia in PCS rats, was also found to be active (Cauli et al., 2007b). The activation of both the ‘normal’ and ‘alternative’ neuronal circuits in hyperammonemic rats led to significantly greater levels of motor output compared to controls, again identifying dysruptions to normal motor functions (Cauli et al., 2007b). Although, hyperammonemia was found to activate the ‘alternative’ circuit, as in PCS rats, it did not completely inhibit the ‘normal’ circuit. This suggests that either other co-occurring factors in liver disease contribute to the disturbances in the neuronal circuitry observed (Cauli et al., 2007b), as with hippocampal LTP, or the levels of hyperammonemia reached in rat models of chronic hyperammonemia, are not great enough to provide the complete inhibition of the ‘normal’ circuit (Cauli et al., 2007b). Both explanations are plausible, recently PCS rats were reported to have three fold greater ammonia levels in their brain tissues than in rats with chronic hyperammonemia (PCS rats, 1.8 μmol/g tissue; Hyperammonemia rats, 0.55 μmol/g tissue; Cauli et al., 2007b). Such increases in brain ammonia levels could be causing the additional dysfunction within the basal ganglia. However, in addition to this, the co-occurrence of inflammation in liver disease could also influence motor activity, as it has been observed that a reduction in inflammation, via the administration of the non-steroidal anti-inflammatory ibuprofen, helps to restore motor function in PCS rats (Cauli et al., 2009b). Consequently, the direct effect of ammonia on neuronal function may not be the only precipitating factor behind motor dysfunction in liver disease states. Despite the implication of other precipitating factors these results demonstrate that mechanisms associated with hyperammonemia can significantly influence the regulation of motor activity, by interfering with the neural control of the basal ganglia-thalamo-cortical pathways.

The evidence from these in vitro and in vivo animal models helps to explain some of the molecular and neural mechanisms behind the functional deficits in learning and memory, and motor processes associated with liver disease and other such hyperammonemic disorders, indicating a strong link between ammonia and deterioration in function. Impairment in learning and memory, and motor activity are evident throughout the whole spectrum of HE severity (Butterworth, 2000; Weissenborn et al., 2001, 2005). In addition to this, the artificial induction of HE by the oral administration of amino acids (AA) in liver disease patients has noted significant repeatable impairment in; memory task performance (Balata et al., 2003; Shawcross et al., 2004), reductions in immediate recall (Balata et al., 2003), and a slowing of reaction times (Oppong et al., 1997; Douglass et al., 2001). Cirrhosis patients also seem to show an increased inability to inhibit motor responses (Bajaj et al., 2007).

Depending on the severity and duration of the associated condition, systemic ammonia concentrations may range from 80 to 300 μmol l⁻¹ in liver disorder patients (Olde Damink et al., 2002). Such concentrations correspond with those commonly observed during fatiguing, intense exercise. Cerebral dysfunction may therefore be present during such forms of exercise where hyperammonemia is present, and hence may contribute to the development of deteriorations in performance commonly observed towards the end of these types of exercise (i.e. fatigue). It is evident that the neurotoxic effects of ammonia can develop rapidly. For example, dysfunction of the NMDA receptor–NO–cGMP pathway can be observed after as little as 15 min exposure to ammonia, albeit in vitro and at the highest concentration (Hermenegildo et al., 1998). In addition, the induction of hyperammonemia via oral AA administration in liver disease patients can show neuropsychological dysfunction within only 2–4 h of administration (Oppong et al., 1997; Douglass et al., 2001; Balata et al., 2003; Shawcross et al., 2004). Finally, the reversal of HE precipitating factors, using treatment strategies such as acetyl l-carnitine (ALC) and l-ornithine phenylacetate (OP) can show a reduction in hyperammonemia and HE symptoms within 1–4 h of administration (Malaguarnera et al., 2006; Davies et al., 2009). This evidence points towards ammonia having the potential to produce significant neurotoxic effects well within the exposure time provided by some forms of fatiguing exercise, hence providing a robust rationale for a link between systemic ammonia accumulation and exercise associated dysfunction and fatigue.

5. Ammonia and fatigue: innocent until proven guilty

Although the ammonia fatigue theory as proposed by Banister is plausible, it currently lacks any strong empirical evidence to support it. The main body of work used by Banister and colleagues to provide support for their theory was one which identified that a reduction in blood ammonia during exercise was accompanied by an increase in exercise performance, in the form of increased exercise duration (Barnes et al., 1964). There were a number of different interventions used to reduce exercise induced ammonia accumulation; training (Barnes et al., 1964), aspartate supplementation (Barnes et al., 1964; Ahlborg et al., 1968) and glutamate supplementation (Brodan et al., 1974). It was suggested that because these interventions showed a reduced accumulation of blood ammonia accompanied by increased exercise capacity, this provided indirect evidence supporting the ammonia fatigue theory (Mutch and Banister, 1983). However, in suggesting this, the other associated mechanisms which may have caused this increased exercise capacity are overlooked. For example, there are major adaptations in both the cardiovascular and skeletal muscle systems as a result of training to consider (Huonker et al., 1996; Coffey and Hawley, 2007).

Nutritional supplementation studies on the other hand, were thought to provide some more robust evidence, as supplements are believed to act on particular metabolic pathways. The studies reported by Banister and colleagues involved both the supplementation of aspartate and glutamate salts (Barnes et al., 1964; Ahlborg et al., 1968; Brodan et al., 1974). It was believed that supplementation with aspartic acid or its potassium–magnesium salts, would increase plasma availability of aspartate for ureagenesis in the liver, enhancing ammonia removal via this process (Barnes et al., 1964). In addition, increased plasma aspartate is thought to stimulate transaminase reactions, leading to increased oxaloacetate and glutamate formation, thereby providing increased substrate for ammonia detoxification via GS in muscle (Rose et al., 1999). Early research reported both a reduction in plasma ammonia accumulation and an accompanying increase in time to exhaustion in rats (Barnes et al., 1964) and humans (Ahlborg et al., 1968) after potassium–magnesium aspartate supplementation, thereby suggesting a beneficial effect of such supplementation on delaying ammonia induced fatigue. However, even with more recent studies showing similar beneficial effects of combination aspartate supplementation (aspartate, asparagine and carnitine) in rats (Lancha et al., 1995), other recent literature have failed to identify similar findings in humans, with no
Although the effects of ammonia on CNS function are a major component to the rationale for the ammonia fatigue theory, ammonia's effects within the periphery were also considered, as a concentration dependent decrease in muscle contractility and twitch tension had been observed in isolated frog muscle exposed to ammonium ions (Heald, 1975). With the concentration of ammonia in muscle vastly exceeding that which ever reaches other organs in the body during exercise (Muscle ammonia concentration ~0.4–1 mM; Katz et al., 1986; Snow et al., 2000: Plasma ammonia concentration ~70–190 μM; Nybo et al., 2005; Dansgaard et al., 2004). This evidence suggests that the accumulation of ammonia in muscle may contribute to ammonia induced fatigue by directly affecting muscle contractility. However, this interpretation is not without limitation as a recent study has identified that in vitro diaphragm muscle contractility, fatigue rate and recovery are only affected by exposure to supraphysiological concentration of ammonia (greater than 5 mM). No effect was observed at physiologically relevant ammonia concentration ranges of 0.11–5 mM (Shanely and Coast, 2002). This supports other earlier findings, which also found no significant effect of ammonium ions (2 mM concentration) on rat hind limb muscle contractile properties (Stephenson and Stephenson, 1996). Therefore, the idea of a significant peripheral effect of ammonia seems unlikely during exercise, due to the lack of an effect at physiological concentrations.

Since the publication of the original ideas by Banister and colleagues, few researchers have attempted to fully substantiate this theory. Of the studies which have been performed, the majority have either investigated the production and metabolism of ammonia within the periphery (Spodaryk et al., 1990; Graham et al., 1993, 1995b; Rush et al., 1995; Esbjornsson-Lijedahl and Jansson, 1999; Snow et al., 2000; Mohr et al., 2006), or investigated the link between attenuation of ammonia production during exercise and performance (Denis et al., 1991; Eto et al., 1994; Carvalho-Peixoto et al., 2007; Bassini-Cameron et al., 2008). In light of this, it is unsurprising that the ammonia fatigue theory has not remained at the forefront of research interest. However, the findings presented in some more recent literature may warrant the link between ammonia, the brain and fatigue to be revisited.

5.1. Recent progress?

One of the major failings of many fatigue theories, which derive a CNS origin, is the difficulties encountered when trying to test these theories accurately (Graham et al., 1995a). In vitro studies have improved our understanding of the effects increased levels of ammonia may have on cellular function within the brain. We can theorise from these what may be happening in vivo, to cause the development of fatigue centrally in the exercising human. However, we encounter problems if we want to directly test these theories in humans due to the difficulties in accessing the CNS, and as such this can lead to the almost unavoidable consequence of making a number of assumptions. This has been the case in much of the ammonia fatigue studies to date with a heavy reliance on the link between peripheral ammonia accumulation and an accompanying deterioration in performance as the mainstay of its evidence. Making the assumption that peripheral ammonia accumulation will reflect central ammonia accumulation is often believed to not be sensible due to the inconsistencies which have been reported in a number of clinical correlation studies (Ong et al., 2003; Kundr et al., 2005). Recently the use of 13N positron emission tomography (PET) in pathology has identified that the cerebral trapping of 13N labelled ammonia, is similar in both cirrhotic patients without HE, cirrhotic patients...
with HE and healthy controls (Keiding et al., 2006a, 2006b; Sorensen and Keiding, 2007). This finding suggests that brain ammonia kinetics may be similar in diseased and healthy states and that flux of ammonia into brain tissue may be strongly related to arterial concentrations (Sorensen and Keiding, 2007). This contradicts a number of early studies which have suggested that BBB permeability may increase with disease allowing a greater amount of systemic ammonia as NH₄⁺ into the cerebral tissues leading to the onset of HE, hence explaining the discrepancy in correlation between peripheral ammonia levels and HE severity (Lockwood et al., 1991; Lockwood, 2007). These findings by Keiding and co-workers, would therefore suggest that an increase in peripheral arterial ammonia concentration, which is known to occur during exercise to exhaustion, should lead to an increase in ammonia flux across the BBB into the cerebral tissues and an increased cerebral trapping of ammonia. Once in direct contact with the cerebral tissues, the neurotoxic effects of ammonia may result.

The suggested similarity in ammonia uptake across the BBB which was concluded by Keiding et al. (2006a,b) is based on the assumption that cerebral blood flow (CBF) does not significantly change, which was the case in their study as their subjects were resting throughout. Although there are several studies which suggest that CBF does not significantly change during exercise (Madsen et al., 1993; Nybo et al., 2002, 2005), a recent review highlights limitations to these findings however, because measurement of CBF changes can depend on the method and the position in which the subject is placed during data collection (Secher et al., 2008). The method commonly utilised in studies where no change in CBF has been observed (Madsen et al., 1993; Nybo et al., 2002, 2005), is the Kety-Schmidt technique (Kety and Schmidt, 1948), where sampling takes place from an artery (generally radial for ease of access) and the internal jugular vein after injection of a gas tracer (nitrous oxide or xenon). Arteriovenous difference (a-v diff) of the samples collected then allows for a calculation of CBF via the Fick principle (Kety and Schmidt, 1948). Most exercise studies involve upright exercise in either cycling or leg extension, and during such upright postures the internal jugular vein collapses (Cirovic et al., 2003; Dawson et al., 2004; Gisolf et al., 2004) and the cerebral venous outflow is transferred to the vertebral venous circulation (Valdueza et al., 2000). This will mean that measurements obtained for CBF via the Kety-Schmidt technique may greatly underestimate the true values (Ogoh, 2008; Secher et al., 2008; Ogoh and Ainslie, 2009). In addition, there is asymmetrical drainage of the brain via the two jugular veins, and therefore in order to gain an accurate representation of venous drainage, sampling from both would need to be performed (Ide and Secher, 2000). When CBF is analysed using other methods, such as internal carotid flow (Hellstrom et al., 1996), mean flow velocity as assessed by transcranial Doppler (Doering et al., 1998; Poulin et al., 1999; Gonzalez-Alonso et al., 2004) or ¹³³Xenon clearance (Thomas et al., 1989; Jorgensen et al., 1992a,b), an increase in CBF of approximately 25% during exercise can be observed (Secher et al., 2008). This increase may be dependent on intensity however, as the increase in CBF has been found to level off or even start to drop at exercise intensities greater than approximately 60% VO₂max (Jorgensen et al., 1992a; Hellstrom et al., 1996; Gonzalez-Alonso et al., 2004; Querido and Sheel, 2007).

Such an increase in CBF would therefore have an influence on the findings suggested by Keiding et al. (2006a,b) that healthy controls have a similar ability to take up and trap ammonia, as in cirrhosis. Cerebral uptake is a function of CBF,

Cerebral uptake = CBF x a-v diff (Ott and Larson, 2004) (6) therefore with an increase in CBF, we can assume that cerebral uptake will also increase, via an increase in delivery to the brain (Ott and Larsen, 2004). Although the extraction fraction (the extraction of the substance from the blood, in this case ammonia, in a single pass) has been shown to decrease with increasing CBF (Raichle and Larsson, 1981), there is a non-linear increase in net extraction (the total amount delivered to the tissues) with increased CBF, because the increase in CBF will compensate for the decrease in single pass extraction (Phelps et al., 1981). It can be assumed, therefore, that although at rest the flux of ammonia across the BBB is likely similar in healthy individuals and cirrhotic patients (Keiding et al., 2006a,b). When exercise is initiated in healthy subjects and CBF increases, this similarity ceases and healthy subjects should have a greater ability to take up and trap ammonia in the cerebral tissues. This would mean that any increases in the arterial concentration of ammonia during exercise, would create an environment whereby flux of arterial ammonia into the brain could in fact be greater than the equivalent concentrations in diseased individuals.

To our knowledge there are only two studies, to date, which attempt to directly investigate cerebral uptake of ammonia during exercise (Dalsgaard et al., 2004; Nybo et al., 2005). Both Dalsgaard et al. (2004) and Nybo et al. (2005) used a-v difference and cerebrospinal fluid (CSF) ammonia concentrations to draw conclusions regarding cerebral ammonia metabolism during exercise. Although the methods and exercise protocols used were different; Nybo used a prolonged (2–3 h duration) submaximal (~60% VO₂max) cycling protocol performed by both well-trained and less well-trained subjects, whereas Dalsgaard used an incremental whole body (arm and leg) cycling protocol to exhaustion (9–16 min duration) in normal healthy adults. Both studies were able to identify evidence of an increased cerebral uptake of ammonia during exercise (Dalsgaard et al., 2004; Nybo et al., 2005). The study by Nybo et al. (2005) included an additional variable of glucose supplementation, however, for the purposes of this review only the results of the without glucose supplementation trials will be discussed.

Nybo et al. (2005) found that after completion of the submaximal cycling protocol, mean arterial ammonia concentrations were 56 ± 13 and 190 ± 44 μM in the well-trained and less-trained groups respectively. Although this only represented a modest increase in arterial concentration in the well trained group, there was still a significant shift in cerebral ammonia uptake, from a net balance of zero at rest and after 30 min of exercise, to a positive uptake of 3.7 ± 1.3 μmol min⁻¹ after 3 h of exercise (Nybo et al., 2005). Cerebral uptake was not measured in the less-trained group; however, there was a significant increase in CSF ammonia levels, indicating that exercise in this group of subjects was also associated with cerebral uptake and accumulation of ammonia. Dalsgaard et al. (2004) also reported an a-v difference for ammonia of 17 μmol l⁻¹ due to the shorter, more intense exercise bout, indicative of cerebral uptake. The authors did not measure CBF, but assumed a CBF of 0.7 l min⁻¹ for their subjects, based on previous findings (Dalsgaard et al., 2004). Therefore, using this information and equation 6, an estimate of cerebral ammonia uptake can be made for their data to allow comparison with Nybo et al. (2005). Nybo et al. (2005) used a value for cerebral plasma flow (CPF) to calculate cerebral uptake of ammonia instead of CBF, CPF is CBF corrected for plasma volume based on the haematocrit. Using an assumed median plasma volume of approximately 55% (Thirup, 2003), and the data from Dalsgaard et al. (2004), CPF may be estimated as 0.385 l min⁻¹. Accordingly, cerebral uptake during this exercise bout would have been 6.5 μmol l⁻¹ min⁻¹, which is greater than that reported in the prolonged exercise bout in the study by Nybo et al. (2005). Although a high cerebral uptake of ammonia was suggested by these findings, Dalsgaard et al. (2004), unlike Nybo et al. (2005), did not report increases in CSF ammonia levels to accompany this ammonia uptake. This therefore suggests that removal capacity was sufficient during
This new evidence to support the uptake and accumulation of ammonia in the cerebral compartments during exercise, suggests that it may be time to revisit the ammonia fatigue theory. The results provided by Nybo et al. (2005) and Dalsgaard et al. (2004) shows evidence of ammonia accumulation in the cerebral tissues, but it is not known whether this accumulation is a significant enough process to induce any of the neurotransmitter and cellular dysfunction linked to disease states (Felipo and Butterworth, 2002). There is evidence from animal models that cerebral ammonia levels during prolonged exercise may be associated with interference in neurotransmitter metabolism, i.e. the concentrations of the excitatory and inhibitory neurotransmitters glutamate and GABA (Guezennec et al., 1998). However, there is no evidence for this in humans yet. Ammonia may play a significant role in fatigue during exercise through more subtle interactions, possibly mimicking some of the effects observed in hyperammonemic disease states and animal models (Aguilar et al., 2000; Balata et al., 2003; Shawcross et al., 2004; Mendez et al., 2009). The effects of ammonia seem to be at their greatest within the brain areas involved in learning, memory and motor activity (Casli et al., 2008a; Monfort et al., 2009). Most fatigue theories concern the body’s ability to keep exercising and maintaining power output for longer. It seems however, that there may be other psychological aspects of fatigue that are often overlooked, such as perception for action, decision making and motor control. There may well be the need to maintain exercise capacity in many competitive sports in order to succeed, however there is also the need to maintain a high level of decision making (Smeeton et al., 2005).

5.2. A new perspective for ammonia in exercise?

There have been many publications (>200; Brisswalter et al., 2002) reporting a relationship between physically demanding/exhausting exercise and mental performance. However, the findings still remain equivocal, due to the different strategies and interventions used to test this relationship.

In the majority of studies where acute bouts of exercise have been implemented an improvement in the cognitive measures is often observed (Brisswalter et al., 2002; Tomporowski, 2003; Davranche and Audiffren, 2004; Davranche et al., 2006). Much of the present literature supports the hypothesis proposing an energizing or arousing effect of exercise on information processing (Audiffren et al., 2008). This effect may be a direct or indirect consequence of exercise-induced increases in the neurotransmitters epinephrine and norepinephrine (Brisswalter et al., 2002; Tomporowski, 2003). Suggesting that acute or intense exercise, acts in a similar way to psychostimulant drugs by priming the individual to respond rapidly to incoming sensory information through improved allocation of attentional resources (Tomporowski, 2003). This mechanism is by no means conclusive, as recent findings have shown (McMorris et al., 2008). Although the exercise used in these studies is often of a moderate to intense nature, during which fatigue is likely to develop along with an accompanying increase in systemic ammonia. The duration of the intervention tends to be quite short (no more than 20–30 mins) and this may not provide sufficient time for the accompanying rise in ammonia to have any significant effects on cerebral functioning. Indeed from the results of Dalsgaard et al. (2004) and Nybo et al. (2005), CSF ammonia levels were not found to be significantly increased (Dalsgaard et al., 2004), and cerebral uptake of ammonia did not change (Nybo et al., 2005) within a similar time period of less than 30 min. The buffering capacity of the brain for ammonia, in terms of glutamine synthesis, was not exceeded by this duration of exercise. The stimulatory effect exerted by increased arousal and catecholamine release during such exercise may also mask any effects on neural function or metabolism which may be beginning to take place due to a rise in ammonia. Furthermore, the removal of the exercise stress after only a short duration, may quickly reverse any effect and it will not be captured by post exercise tests of cognition.

With longer duration exercise, however, other effects are often observed. It is evident that submaximal prolonged exercise (where duration can exceed 90 min) can produce contrasting results to shorter duration or more intense exercise. Cognitive processes have been found to be significantly compromised, with impairment in both short-term memory and motor control processes after 2 h of submaximal exercise (Cian et al., 2000). Furthermore, Grego et al. (2004) found that after 2 h of submaximal exercise, there were significant changes in the P300 component of the event-related brain potential (ERP), an electrophysiological measure using EEG, which is believed to represent the cortical neural activity underlying cognition (Polich and Kok, 1995). Short latencies and large amplitudes within the P300 ERP have been related to higher levels of basic cognition and superior memory, respectively (Polich, 2007). Grego et al. (2004) noted that the P300 latency increased significantly after 2 h of submaximal exercise, suggesting that basic cognition may be affected in a negative manner by exercise stress which exceeds this duration (Grego et al., 2004). Complementary to this, P300 amplitude also started to decrease after 2 h, which may suggest that immediate memory processes are also starting to be reduced, however this was not found to be statistically significant (Grego et al., 2004). A more recent case study of an ultra-endurance athlete lends some support to these findings, with increases in P300 latencies and reductions in P300 amplitudes when analysed during a 24-h foot race (Doppelmayr et al., 2007). Unfortunately, the original work by Grego et al. (2004) reported no behavioural data to accompany their ERP measures and as such it is difficult to determine whether the observed changes in P300 latencies are representative of changes in cognitive performance. However, a follow-up study by the same group did identify a reversal of improvements in cognitive task performance beyond 2 h of the same type of exercise (Grego et al., 2005).

Even though there is little research in this area, from the available data, it seems plausible to propose that prolonged exhausting exercise may eventually lead to deteriorations in cognition where duration exceeds 2 h. But what could be causing this to happen, when for the previous 2 h of exercise there was a facilitation of cognition? The onset of dehydration has been proposed as one potential cause (Cian et al., 2000, 2001). However, Grego et al. (2005) reported no difference in the effect of exercise on cognitive performance when participants were allowed to maintain adequate hydration, and when fluid intake was significantly restricted during 3 h of exercise (Grego et al., 2005). In addition, a more recent study by Tomporowski et al. (2007) has suggested that the exercise induced effects on cognition are independent of hydration status during exercise ranging in duration from 15 to 120 min (Tomporowski et al., 2007). With this in mind could ammonia accumulation be the reason for these deteriorations in cognition observed in exercise greater than 120 min? During such a mode, intensity and duration of exercise, it has been shown that systemic levels of ammonia increase significantly with an accompanying significant accumulation in cerebral compartments (Nybo et al., 2005). Implying, therefore, that ammonia concentrations will reach those capable of exerting influence on cerebral functioning during such exercise. In addition, the main processes which are commonly believed to be affected during such exercise are those also commonly associated with pathological hyperammonemic cerebral dysfunction.
Although in vitro effects of ammonia neurotoxicity have been shown to develop rapidly (Hermengilido et al., 1998), it may take anything up to or beyond 2–3 h before any significant signs of cognitive deterioration are measurable at the behavioural level after the induction of hyperammonemia in liver disease patients (Oppong et al., 1997; Douglass et al., 2001; Balata et al., 2003; Shawcross et al., 2004). If we apply this reasoning to exercise, the effects observed on cognition after very prolonged exercise may be explained. During short duration intense exercise, there are accompanying significant increases in plasma ammonia levels, and an increasing cerebral a-v diff (Dalsgaard et al., 2004) suggesting a large cerebral ammonia uptake during such exercise. Any ammonia that is taken up, however, seems to be efficiently incorporated into the cerebral glutamine pool, as is clear from the absence of an increase in CSF ammonia concentration (Dalsgaard et al., 2004). Therefore, it can be assumed that any effects of ammonia on cerebral function due to this uptake will be limited. In contrast, during prolonged exercise, although the increase in ammonia concentration is more gradual, levels can match or even exceed those of intense exercise (Brouns et al., 1990). With prolonged exposure to such levels, uptake across the BBB will eventually exceed detoxification by the astrocytes, leading to a significant cerebral accumulation of ammonia (Nybo et al., 2005). Such ammonia accumulation should lead to similar disturbances in both neuronal and astrocyte mechanisms as those observed in vitro. As a result the cognitive disturbances, which seem to develop after prolonged durations of submaximal exercise, may be triggered. Ammonia therefore, unlike what was previously thought, may have a far more subtle, but significant, role to play in the development of fatigue. Ammonia may only have a significant impact during exercise of prolonged duration, where its influence may be limited to mild disturbances in cognition. Therefore, negative effects would result from tasks that have a significant cognitive component or be observed in individuals that choose to perform them that way, rather than directly affecting the maintenance of power output or efficient muscle contraction.

6. Conclusions

It seems likely that ammonia accumulation plays a subtle role during exercise. It is likely that its influence on exercise may mimic the effects observed in liver disease patients with minimal or low grade HE, where the onset of hyperammonemia leads to subtle changes in cognition (Balata et al., 2003). It is the relatively gradual onset of these effects over a period of 2–3 h after hyperammonemia onset, which implicates ammonia and its subtle influences on cognition with exercise and fatigue. As during prolonged submaximal exercise of a similar duration (greater than 2 h), we tend to observe the beginnings of mild cognitive disturbances as evident through both behavioural and neurophysiological measures (Grego et al., 2004, 2005; Doppelmayr et al., 2007). Such changes coincide with pronounced increases in systemic ammonia concentrations, similar to those reported in liver disease, in addition to a positive cerebral uptake and accumulation (Nybo et al., 2005). Therefore, combining what is now known about the pathophysiology of ammonia through in vitro studies, this tends to suggest that the onset of cognitive disturbances during prolonged exercise may in fact be being caused, at least in part, by ammonia accumulation, and that ammonia build-up is less involved with physical performance but more involved with deficits in cognition, decision-making and skilled performance. The rapid development of minimal and non-invasive imaging procedures may be key to providing direct evidence for this theory, as methods such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scanning become more reliable, accurate and accessible (Boecker et al., 2008; Jantzen et al., 2008; Tashiro et al., 2008), allowing a more direct assessment of the role ammonia plays in fatigue to be performed. The application of such imaging techniques with manipulation of systemic ammonia levels, by exercise or other methods, may prove useful in discovering more about links between fatigue, ammonia and the brain. As it seems the original question proposed by Tashiro over 80 years ago: “Is there a relationship between NH3 production and fatigue...?” (Tashiro, 1922, p. 542), still remains to be answered.

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