

Brain-barrier Regulation, Metal (Cu,Fe) Dyshomeostasis and Neurodegenerative Disorders in Man and Animals.

Susan Haywood

Institute of Infection and Global Health, School of Veterinary Science, University of Liverpool, ic2 Science Park, Brownlow Hill, Liverpool L3 5RF

Corresponding author: Dr Susan Haywood

Email: shaywood@liverpool.ac.uk

Abstract

The neurodegenerative diseases (Alzheimers, Parkinsons, amyotrophic lateral sclerosis, Huntingdons) and the prion disorders, have in common a dysregulation of metalloprotein chemistry involving redox metals (Cu,Fe,Mn). The consequent oxidative stress gives rise to protein plaques and neuronal cell death. An equilibrium exists between the functional requirement of the brain for Cu and Fe and their destructive potential with the production of reactive oxygen species. The importance of the brain barrier is highlighted in regulating the import of these metals. Upregulation of key transporters occurs in foetal and neonatal life when brain metal requirement is high and is down-regulated in adult life when need is minimal. By contrast a neonatal mode of CTR1 upregulation persists in feral N.Ronaldsay sheep. This has led to the premise that metal regulation may return to the default setting in ageing with implications for neurodegenerative disease.

Key words: Blood-brain barrier, copper/iron homeostasis, neurodegenerative (Alzheimers, Parkinsons, Prion) disease, North Ronaldsay sheep.

Introduction

The unique structure and organisation of the central nervous system (CNS) makes it particularly vulnerable to changes in its external and internal environment which if unregulated can be associated with neurodegenerative disease. The brain and cord as well as being enveloped and shielded from physical trauma by its meninges and cerebrospinal fluid is also protected from chemical ionic imbalance and uptake of noxious substances by the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCB). However there is evidence that this protective barrier can be compromised during ageing or adverse environmental conditions that may result in the unregulated entry of metals and other toxicants with potential damaging consequences.

The hypothesis of metal-based neurodegeneration is now widely accepted (Barnham and Bush, 2008; Bush and Tanzi, 2008; Bush, 2013) and ageing is a major risk factor. In particular the transition metals, Cu, Fe, (Mn) are essential metals, which have the ability to exist in different ionic states that can provoke the formation of reactive oxygen species (ROS) are especially incriminated. Protein aggregation in the brain is the putative result of oxidative stress with the formation of plaques in Alzheimers disease (AD), Lewy bodies in Parkinsons disease (PD), amyotrophic lateral sclerosis ALS), Huntingdons disease (HD) and the prion diseases (Pr). It is also acknowledged that Cu and Fe increase in the brain as it ages.

The pathobiology of these plaques commands extensive research but less attention is given to why and how this ionic dyshomeostasis occurs in the brain given that the blood brain barrier is tightly regulated. This mini review hopes to rectify in some measure this discrepancy.

1. Metal dyshomeostasis and oxidative stress

Both iron (Fe) and copper (Cu) are essential trace elements for human and animal health and can exist in a variety of oxidation states such as Fe^{++} , Fe^{+++} and Cu^{+} , Cu^{++} . These multiple oxidation states allow the metals to readily participate in oxidation-reduction reactions. Copper is an integral component of various cupro enzymes including cytochrome C oxidase, lysyl oxidase, superoxide dismutase (SOD), tyrosinase, dopamine B-oxidase, and caeruloplasmin. An important function of Fe is to transport oxygen in haemoglobin and myoglobin. Fe is also required for the normal function of cytochrome oxidase, peroxidase and catalase. Furthermore Fe is involved in mitochondrial respiration, DNA synthesis and

biosynthesis of neurotransmitters. Conversely free Fe and Cu ions can readily interact with oxygen to initiate cascades of biochemical reactions leading to the production of free radicals and oxidative stress. Thus a stable homeostasis of Cu and Fe is essential for brain function.

The brain internal milieu is exceptionally susceptible to oxidative stress by toxic reactive oxygen species (ROS) due to its high metabolic demands accounting for up to 20% of all oxygen consumption and its rich concentration of polyunsaturated by fatty acids. [Oxidant stress is regarded as the imbalance between pro-oxidant free radical production and opposing antioxidant defence]. The superoxide radical O_2^- produced disproportionally in the brain mitochondria during cellular respiration undergoes dismutation to hydrogen peroxide (H_2O_2) and thence to water by superoxide dismutase. Alternatively transformation of H_2O_2 in the cytosol may occur in the presence of Cu^+ and Fe^{++} to produce the highly reactive hydroxyl radical $OH \cdot$. Unfortunately the antioxidant defence chiefly in the form of superoxide dismutase (Cu Zn SOD) is easily overwhelmed in the CNS by the high level of oxidising activity in this organ and possible occurrence of the unbound transitional metals Cu^+ and Fe^{++} . These transition metals are essential for life but also inimical if unregulated. Reactive oxygen species (ROS) are responsible for DNA, cell membrane and protein damage. Protein folding can also be a casualty of free radical damage leading to misfolded proteins with aggregation and loss of function. Such plaque formation is a characteristic feature of several degenerative diseases aforementioned. This subject is reviewed in depth by [Gagelli et al \(2006\)](#); [Rivera-Mancia et al, \(2010\)](#).

i. Copper homeostasis

The brain has a high requirement for copper, second only to the liver. On account of its oxidation potential regulation it is necessary to ensure only sufficient but not excess copper is present in the brain. Copper concentrations are maintained at a constant level when maturity is achieved, in human beings 2.9-10.7ug/g wet weight whilst rat brains appear to have a lower

copper content 1-2.3ug/g wet weight (Rongzhu et al, 2009; Gybin, Tkac and Prohaska, 2009; Lutsenko, Bhattacharjee and Hubbard, 2010). The metal is not uniformly distributed within the CNS. Studies in adult rats show copper enhancement in the medial geniculate nucleus (visual), superior colliculus (motor functions), periaqueductal grey matter (pain and defensive behaviour); also high in lateral amygdala (memory and emotional response) and dorsomedial diencephalon (thalamus/hypothalamus (Jackson et al, 2006).

Distribution is accomplished by a coordinated system of copper transporters and chaperones which convey copper safely across cell membranes to where it is required. Three major groups are recognised: copper uptake transporters which transfer copper into the cytosol, copper chaperones which facilitate copper distribution to intracellular target organelles, copper transporting ATP-ases which translocate copper into the secretory pathways and small vesicles for the delivery of copper to newly synthesised cuproenzymes. In the first category is recognised high affinity copper transporter (CTR1) in the brain that has high concentrations in the blood-brain barrier (BBB) and blood – cerebrofluid barrier (BCB); also divalent metal transporter (DMT1) which includes the transfer of Zn, Fe, Mn in addition to Cu; secondly CCS or copper chaperone for inclusion of the metal in superoxide dismutase, the major antioxidant in cytosol and mitochondria; copper chaperone Atox1 which transfers copper to the binding sites of the ATP-ases and cytochrome oxidase assembly factors CCO which transfer copper to the terminal enzyme of the respiratory chain in mitochondria. Finally the copper transporting ATP-ases, ATP7A and ATP7B, mutations in which are associated with Menkes disease and Wilsons disease respectively. This subject is reviewed by Lutsenko, Bhattacharjee and Hubbard (2010); Scheiber, Mercer and Dringen, (2014)

Studies on the distribution of the copper transporters and chaperones are in their infancy but it has been shown that ATP7A and ATP7B show cell specific distribution in the adult cerebellum and have distinct enzymatic characteristics and are regulated differently during

development (Barnes, Tsivkovskaia and Lutsenko; 2005). Studies on the human brain have been more limited in deference to its greater size but one study has identified significant relationships between copper transporter levels, CTR1, Atox1, ATPA and B and brain copper content. (Davies et al, 2013).

ii. Iron homeostasis

Fe enters the circulation and is bound to B-globulin apotransferrin to form transferrin which serves as the major vehicle for Fe transport in the body. Upon arriving at the target cells transferrin binds with transferrin receptors (TfR) and through endocytosis is carried into the cell where it is utilised in metabolic processes or conjugated with apoferritin to form the storage protein ferritin.

The brain regulates Fe balance i) via TfR mediated transport or non- mediated transport at brain barriers, ii) storage of Fe as ferritin or iii) efflux of Fe from BCB back into circulation. Alternatively DMT1 can transport Fe across BBB. Fe is primarily transported to the brain parenchyma by BBB and the correlation between TfR density and Fe concentration in various brain regions further suggests that a TfR transport mechanism at the BBB is primarily responsible for the rate of Fe entry into the brain parenchyma (Zeng and Monnot, 2012). There is evidence for an uneven distribution of TfR receptors in cerebral capillaries may be responsible for the uneven distribution Fe in brain regions eg >Fe in striatum and hippocampus (Deane, Zheng and Zickovic, 2004).

Excess Fe exits the brain from the BCB via DMT or Tf R (Zheng and Monnot, 2012).

Iron dysregulation has a major role in PD also in AD, and is implicated in amyotrophic lateral sclerosis, Huntingtons and the prion diseases. All these diseases link similar mechanisms oxidative stress, protein aggregation and mitochondrial dysfunction (Belaidi and Bush, 2016).

2. The brain barrier system and the regulation of metal homeostasis.

The overall concentration of copper and iron, and their compartmentalisation within the mature brain, must be maintained to maintain optimum activity with minimum oxidative damage and ensuing disease. Ageing and the onset of related disease has been associated with an increase in internal copper content and iron and discompartmentalisation (Lutsenko.Bhattacharjee and Hubbard, 2010; Belaidi and Bush, 2016).

Probably the most important system for maintaining overall copper and iron homeostasis is the brain barrier which it has been suggested becomes more permeable during ageing due to a variety of developmental and genetic factors (Zheng 2001). The brain barrier is a protective system consisting of the blood - brain barrier BBB and the blood-cerebro fluid barrier BCB which regulates these metals entering and leaving the brain (Zheng and Monnot, 2012).

The sequence of events that takes place in this process is currently better understood with regard to copper than for iron and the argument will accordingly take account of this in the following account ;-

The BBB exerts a selective discrimination for copper at the level of the cerebral microvascular endothelium. This consists uniquely of non-fenestrated capillary endothelium that with adhering pericytes and astrocyte end feet on the abluminal surface, constitutes a unique neurovascular unit (Hawkins and Davies, 2005]. Astrocytes are considered an integral feature of copper homeostasis in the brain since it has been shown that excess copper is stored in these cells bound to metallothionien (Haywood et al, 2008; Tiffany-Castiglioni, Hong and Qian, 2011; Haywood and Vaillant 2014). Furthermore the strategic localisation of astrocytes makes them ideally positioned to regulate the transport of copper from the BBB to neuronal cells (Scheiber and Dringen, 2012). The BBB is concerned mainly with copper influx mediated by CTR1 (Zeng and Monnot;2012; Scheiber, Mercer and Dringen, 2014;

Haywood and Vaillant, 2014) from which it is transferred via ATP7A directly into the brain for neuronal activities (Zheng and Monnot, 2012) or taken up by astrocyte end feet, stored and later released into neurons as required (Scheiber and Dringen, 2012).

The BCB is located in the choroid plexus, a polarised and highly vascularised organ in the roof of the brain ventricles. The BCB comprises a single layer of tightly appositional epithelial cells facing the cerebrospinal fluid CSF, underlying connective tissue and an inner layer of capillaries lined by fenestrated endothelial cells. The function of the BCB in addition to secreting CSF is concerned with the removal of excess copper and certain other substances from the CSF (Zeng, Aschner and Gersai-Egea, 2003).

Ontological development of brain barrier

It is important to recognise that the functional behaviour of the brain barrier is not necessarily fixed throughout life. The ontogenesis of the brain barrier is rarely discussed, although it is recognised that the BBB is immature at birth and for some time thereafter in several species (Zheng, 2001). This is often referred to as 'leaky', an unfortunate description since research has shown that functionally developed transport mechanisms are well developed in the embryonic and neonatal brain (Saunders, N. R.; Liddelow, S. A. and Dziegielewska, 2012; Saunders et al, 2018).

Regulation of the brain barrier (BBB and BCB) is strictly controlled and operates within defined time-related functional domains in the mammalian brain eg foetal/neonatal and postnatal. Each of these domains is structurally and physiologically adapted to the particular life stage of the organism. During the developmental stage i.e the foetal and neonatal periods, the brain is growing rapidly and there is a high demand for especially copper and iron, but which in the adult is no longer required.

Regulation of divalent metals takes place at the brain barriers which shield the brain from ionic perturbations in the blood. These barriers consist of tight junctions at the blood-brain interface and the blood-cerebrospinal fluid interface which are present from a very early stage in embryonic development and act as a diffusion restraint. The transfer of essential nutrients into (ingress) and out of (egress) the brain is by means of specific energy-dependant transporters.

The key junctional genes are upregulated and expressed as required. Transport mechanisms in the BBB and BCB of the developing brain which determine the internal concentration of trace metals Cu, Zn, Mn and Fe are up-regulated early in foetal life continuing into neonatal life when demand is high in the growing brain but down regulated when the brain is mature. Indeed some metal transporters which include copper are more active during development than in the adult in reflection of the greater needs of the brain for copper during intrauterine life (Saunders, Liddelow and Dziegielewska, 2012). On reaching maturity the requirement for copper by the adult brain is reduced and a down regulation of the main copper transporter CTR1 would be expected. A breakdown in this regulatory sequence can result in metal dyshomeostasis with adverse consequences.

Persistence of neonatal copper uptake in North Ronaldsay sheep.

A study of brain barrier regulation in North Ronaldsay sheep, a feral breed which display a unique copper metabolism - of use to these sheep in their Cu deplete, niche environment (Fig1) has illustrated this most strikingly (Haywood, S. and Vaillant, 2014). There is a persistent overexpression of the copper transporter CTR1 in the capillaries of the BBB in adult life in North Ronaldsay sheep; this allows for an enhanced uptake of copper into the brain (Fig 2) unaffected by levels of dietary exposure. Whilst this has proved advantageous to the sheep in a copper deplete environment the persistence of the neonatal mode of copper

regulation can have deleterious consequences if the sheep should be subjected to copper challenge.

It is speculated from this particular study that the elevated copper content of the ageing (human) brain may derive from a dysregulation of CTR1 at the brain barrier with age and a return to the default (foetal/neonatal) setting with implications for neurodegenerative disease (Haywood, S. and Vaillant, 2014). The reasons for this must currently remain speculative but may involve changes in the genetic control which may be innate or possibly due to environmentally induced mutation. Certainly a study into the control of brain-barrier regulation is well overdue.

4. Neurodegenerative disorders

Neurological disorders of cognitive and sometimes motor processes are characterised by the presence of misfolded proteins causing neuronal damage. Such aberrant proteins have a typical tendency to form solid deposits or aggregates such as the plaques in Alzheimers disease (AD), Lewy bodies of Parkinsons disease (PD), the Bunini bodies of familial amyotrophic lateral sclerosis (ALS), inclusions of Huntingdons disease (HD), and lastly the plaques of the prion diseases. All these disorders have a common endpoint usually involving metals and are collectively known as neurodegenerative diseases.

Proteins are the major component of cells and are involved in nearly every biological process taking place. Their functionality depends on their ability to fold into 3D structures with intermediate stages in the folding process playing significant roles in the translocation of substances and trafficking to specific cellular locations. Whereas increased Cu and Fe have been associated with the diseases of ageing such as AD, ALS, PD, and HD, certain other diseases such as the prion diseases occur over a wider age range in man and animals. All of these disorders are associated with cognitive and in some cases motor disturbances and have

one thing in common in that they have intracellular aggregates which are the outcome of the deposition of misfolded protein. Whilst a few diseases have a genetic component there is widespread consensus that the protein aggregation in neurodegenerative disorders is largely determined by the impaired control of free radicals involving metal ions.

It is currently realised that oxidative stress is the chief cause of protein aggregation in the ageing brain and that this is the outcome of impaired copper, iron (and possibly other metals) homeostasis. There are two generic reactions of relevance to neurodegenerative disease 1.a metal-protein association leading to aggregation and 2.a metal catalysed protein-oxidation leading to protein damage and denaturation. This subject is reviewed by [Gagelli et al, \(2006\)](#) and [Rivera-Mancia et al, \(2010\)](#).

Ageing and some disease states are associated with elevated brain copper (Cu) and other metals, notably iron (Fe). The brain barrier likewise and other internal barrier systems become more permeable allegedly due to ageing, genetic or environmental influences affecting metal compartmentalisation. AD, PD, ALS, HD have all been associated with raised metals chiefly Cu (Zn) and Fe. The prion diseases which include Creutzfeldt –Jacob disease in man and in animals bovine spongiform encephalopathy, scrapie in sheep are all associated with brain copper dyshomeostasis and in the latter possible displacement of Mn.

i. Alzheimers disease

Alzheimers disease (AD) is associated initially with memory loss, a decline in cognitive function and ultimately a common endpoint involving metal dyshomeostasis. Extracellular senile plaques of amyloid-AB polypeptide and intracellular neurofibrillary tangles are diagnostic hallmarks ([Scheiber, Mercer and Dringen, 2014](#); [Rivera-Mancia et al, 2010](#)). The AB peptides are generated from the amyloid precursor protein (APP) a membrane protein widely distributed in the brain of unknown function.

Whilst the plaques have attracted most attention increasingly they are seen as secondary to metal dyscompartmentalisation. The plaques are copper enriched (to the overall detriment of the brain generally) and this has given rise to a “metal only” hypothesis. (Belaidi and Bush, 2016; Bush and Tanzi, 2008). Indeed the role of copper dyshomeostasis in AD has been further emphasised in that it has been postulated that an ‘epidemic of AD’ has been seen in developed countries which have nearly universal copper piping, aided by copper supplements and a high fat component in the diet (Brewer,2012).

More recently AD metal dyshomeostasis has extended to include Fe which has been observed surrounding the plaques and it has been suggested that it is the combination of redox metals (Fe and Cu) in the vicinity of plaques which is responsible for the neurotoxicity of the AD plaques. (Belaidi and Bush,2016).

ii. Parkinsons disease is the second most prevalent neurodegenerative disease world-wide characterised by motor disturbances tremor, rigidity and bradykinesia. The pathological changes of PD include degeneration and death of dopinergic neurons within the substantia nigra pars compacta (SN) and the accumulation most common neurodegenerative disease in humans with no animal homologues apart from genetically-contrived lab animal models; this is predominantly a disease of advanced age, although a genetic component is postulated (Rivera-Mancia et al, 2010). The selective molecular basis of PD is not entirely clear but a scientific consensus suggests multiple factors are involved in the degenerative process which include oxidative stress, inflammation, protein degradation with the aggregation of synuclein.. There is also recognition between the occurrence of PD and industrialisation with further the implication of heavy metal involvement (Fe, Cu and Mn). More recently the role of Fe has been recognised with the involvement of Fe transporters DMT1 and ferroportin, but with strong and growing evidence for Cu and Zn miscompartmentalisation (Rivera-Mancia et al, 2010). Moreover the central role of Fe in PD has become increasingly emphasised. Fe

increases in the brain during normal ageing and disproportionately so in the substantia nigra (SN). Dopamine is initially chelated by neuromelanin in SD but increasingly is overwhelmed by accumulating Fe which initiates the pathophysiology of PD (Zucca et al, 2018) that leads to neuronal cell death of dopinergic neurones and the accumulation of intracellular inclusions designated Lewy bodies (Rivera-Mancia et al, 2010; Belaidi and Bush,2016).

Therapeutic intervention using Iron chelation is currently the most useful strategy, using Deferiprone, a recognised treatment for Fe overload disorder (Belaidi and Bush, 2016).

Proactively in the long run it would seem that with all neurodegenerative disorders an understanding of the genetic and environmental influences which determine the movement of metals into and out of the brain would be a most useful approach.

iii Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease clinically manifested by weakness and wasting of affected muscles mediated by loss of motor neurons of the anterior horns of the spinal cord. ALS is in the main sporadic (90%) but does possess a familial genetic (autosomal dominant) component (FALS) in another 10%. Both sporadic ALS and FALS are characterised by the malfunction of Cu-Zn SOD with loss of antioxidant function and misfolding of the protein resulting in cytoplasmic aggregates, Bunini bodies and Lewy body-like inclusions. Superoxide dismutases are the major antioxidant scavenging enzymes with Cu ion as the catalytic function and Zn ion that maintains structure. See reviews (Rivera-Mancia et al, 2010) on the association between FALS and SOD1 mutation. Furthermore ‘the diminished antioxidant capacity of the motor cell is further aggregated by the SOD gain of toxic function in which Cu bound to the protein plays a central role. Additionally involvement of SOD in regulating Fe proteins plays an important role in Fe accumulation complicating oxidative stress.’ (Rivera-Mancia et al, 2010).

iv. Huntingtons disease is an autosomal dominant neurodegenerative disorder characterised by progressive motor, cognitive and psychiatric deterioration with neuronal loss widespread. Disruption in Fe homeostasis, free radical generation and protein precipitation are all features with once more increasing evidence of an as yet unexplored disturbance of copper metabolism. (Scheiber, Mercer and Dringen 2014; Rivera-Mancia et al, 2010).

All the evidence indicates disturbances of metal functioning (Cu and Fe) with metal transporters and altered compartmentalisation implicated. Mechanisms of damage elicited by Cu and Fe common to AD, PD ALS and HD include free radical production, protein aggregation and metal transport alteration. All this suggests miscompartmentalisation of metals which in turn may implicate genetic regulatory regulation.

v. Prion disease

A final major category of metal associated neurodegenerative disease are the Prion disorders which have crossed the species boundary and are recognised in man as Creutzfeldt Jacob disease (CJD) in man, with more recently a variant form (vCJD) in young people. In animals the prion disorders are given the generic name of transmissible spongiform encephalopathies (TSE's) and are recognised in cattle as bovine spongiform encephalopathy (BSE), scrapie in sheep (PrP^{Sc}) and recognised latterly transmissible mink encephalopathy (TME), chronic wasting disease in mule deer (CWD), and reports of TSEs in zoo animals and felines (Haywood and Brown, 2003). They have similarities with AD and the other neurodegenerative disorders in that they all show protein aggregation or plaques as a dominant part of their pathology which consist of an abnormal protease isoform of the prion protein PrP^{Sc}. However they differ from the above in that they may occur in a variety of ages and also that they are associated with a so called 'infectious' agent, the prion protein.

Several decades ago prion disease was limited to sporadic CJD in humans and the exotic rarity Kuru seen in cannibalistic tribes in New Guinea. Now certain point genetic mutations in the prion protein gene are recognised in the familial forms of the disease, Gerstmann-Straussler-Scheinker syndrome, Fatal Familial Insomnia and inherited CJD. In animals scrapie has long been known endemic in UK for >250 yrs and in 1986 Bovine Spongiform Encephalopathy (BSE) was identified in UK cattle, then transmissible mink encephalopathy (TME), chronic wasting in mule deer (CWD) plus reports of TSEs in zoo animals and felines. The national press was alerted when a variant form of CJD vCJD was identified in young people. The supposition at the time was that BSE had crossed the species barrier to infect young people through consumption of beef products though this was never proven. All these diseases are progressively fatal degenerative disorders of the CNS and a pathology consisting of spongiform changes in the neurons together with the deposition of large amyloid plaques. (Brown,2001;Brown, 2011a and b; Haywood and Brown; 2003).

The cause is the prion protein PrP which is a normal component of the nervous system and found in the cell wall and is a copper-containing protein with SOD like activity. The infective form PrP^{sc} has been shown to be a Mn-substituted isoform which is protease resistant and lacks antioxidant capacity. It is not known how the change occurs and is thought possible that Mn from enriched soils is responsible for the metal substitution in herbivores and that a concentration of the isoform provokes self- replication in that it recruits normal PrP in a hijacking of the synthetic machinery which then operates in an uncontrollable fashion. A unique distinguishing feature of the prion disorders or TSEs is that despite lacking nucleic acid they can spread to another host by lateral spread, ingestion of contaminated tissues and possibly maternal transmission (Brown,2001).

One final intriguing piece of information is that the conversion of PrP^{Cu} to PrP^{Mn} may occur in the astrocyte component of the BBB all of which links the defective brain barrier in the genesis of the metal induced neuropathies described (Brown,2004).

The mechanisms linking metal-induced dyshomeostasis and neuronal disturbance are still unproven but in general these diseases, even the prion disorders, represent a group of neuropathies characterised by a dysregulation of metalloprotein chemistry deriving from defective metal compartmentalisation with protein misfolding, the likely outcome of disordered barrier homeostasis.

Much work needs to be done regarding the pathogenesis of the neurodegenerative disorders but the purpose of this study is to emphasise the importance of the brain barriers and importantly the regulatory changes in the ontogenesis of the blood-brain barrier in ageing, control of which possibly at the genetic level could materially influence the uptake of copper and other redox metals and the genesis of the neurodegenerative disorders.

Acknowledgements

I acknowledge the support of the Royal College of Veterinary Surgeons Trust for a 'Blue Sky' Research Project Award (2008) to myself to study 'Copper Trafficking in the Brain of the North Ronaldsay sheep: a Model for the Study of Neurodegenerative Disease.'

References

- Adlard, P.A.; Bush, A.I. Metals and Alzheimers Disease. *Journal of Alzheimers Disease*, **2006**, *10*, 145-163.
- Barnes N, Tsivkovskaia N, Lutsenko S. The copper transporting ATP-ases, Menkes and Wilson disease proteins have distinct roles in adult and developing cerebellum. *J. Biol. Chem.* **2006**, *10*, 9640-9645.
- Barnham, K. J.; Bush, A. I. Metals in Alzheimer's and Parkinson's diseases. *Current Opinion in Chemical Biology*, **2008**, *12*, 222-228.
- Belaidi, A.A. and Bush, A.I. Iron neurochemistry in Alzheimers disease and Parkinsons disease: targets for therapeutics. *Journal of Neurochemistry* **2016**, *139(suppl.1)* 179-197.
- Brewer, G. Copper toxicity in Alzheimers disease: cognitive loss from ingestion of inorganic copper. *Journal of Trace Elements in Medicine and Biology* **2012**, *26*, 89-92.
- Brown, D. R.(a) Metals in neurodegenerative disease. *Metallomics*, **2011**, *3*, 226-228.
- Brown, D. R.(b) Prions and manganese: a maddening beast. *Metallomics*, **2011**, *3*, 229-23.
- Brown, D. R. Copper and prion disease. *Brain Research Bulletin*, **2001**, *55 (2)*, 165-173.
- Brown, D. R. Role of the prion protein in copper turnover in astrocytes. *Neurobiology of Disease* **2004**, *15*, 534-543.
- Bush, A. I.; Tanzi, R. E. Therapeutics for Alzheimers disease based on the metal hypothesis. *Neurotherapeutics* **2008**, *5*, 421-432.
- Bush, A. I. The metal theory of Alzheimers disease. *Journal of Alzheimers Disease* **2013**, *33*, S277-S281.
- Davies, K.M, Hare, D.J, Cottam, V, Chen. N, Hilgers. L, Halliday.G, Mercer, J.F.B. and Double, K.L. Localisation of copper and copper transporters in the human brain. *Metallomics* **2013**, *5*, 43-51.
- Deane R, Zheng W and Ziokovac B. V. Brain capillary endothelium and choroid plexus endothelium regulate transport of transferrin bound and free iron into rat brain. *J. Neurochem* **2004**, *88*, 813-820.
- Gagelli, E. Kozlowski, H. Valensin, D. Valensin , G. Copper homeostasis and neurodegenerative disorders (Alzheimers, Prion and Parkinsons diseases and Amyotrophic lateral sclerosis) *Chem Rev.* **2006**, *106*, 1995-2044.
- Gybina, A. A.; Tkac. I. and Prohaska, J.R. Copper deficiency alters the neurochemical profile of developing rat brain. *Nutr. Neurosc.* **2009**, *12(3)*, 114-122.
- Hawkins, B. T.; Davies, T. P. The blood-brain barrier/ neurovascular unit in health and disease. *Pharmacological Reviews* **2005**, *57*, 173-185.

- Haywood, S.; Brown, D. R. Transmissible Spongiform Encephalopathies. *Veterinary Times* **2003**, *33* (2), 8-10.
- Haywood, S.; Paris, J.; Ryvar, R.; Botteron, C. Brain copper elevation and neurological changes in North Ronaldsay sheep: a model for neurodegenerative disease? *J. Comp. Path.* **2008**, *139*, 252-255.
- Haywood, S. and Vaillant, C. Overexpression of copper transporter CTR1 in the brain barrier of North Ronaldsay sheep: Implications for the study of neurodegenerative disease. *J. Comp. Path.* **2014**, *150*, 216-224.
- Jackson, B.; Harper, S.; Smith, L. and Flinn, J. Elemental mapping and quantitative analysis of Cu, Zn, and Fe in rat brain sections by laser ablation ICP-MS. *Anal. Bioanal. Chem.* **2006**, *384* (4), 951-957.
- Lutsenko, S. Bhattejee, A. Hubbard, A. Copper handling and machinery of the brain (Critical review) *Metallomics* **2010**, *2*, 596- 608.
- Rivera-Mancia, S.; Perez-Neri, I.; Rios, C.; Tristan-Lopez, L.; Rivera-Espinosa, L.; Montez, S. The transition metals copper and iron in neurodegenerative disease. *Chemico-Biological Interactions* **2010**, *186*, 184-199.
- Rongzhu, L.; Suhua, W.; Guangwei, X.; Chunlan, R.; Fanghan, H.; Junjie, J. and Aschner, M. Zinc, copper, iron, and selenium levels in brain and liver of mice exposed to acrylonitrile. *Biol. Trace Elem. Res.* **2009**, *130* (1), 39-47.
- Saunders, N. R.; Liddelow, S. A.; Dziegielewska, K. M. Barrier mechanisms in the developing brain. *Frontiers in Pharmacology* **2012**, *3*, article 46, 1-18.
- Saunders N. R, Dziegielewska, K. M, Mollgard K. and Habgood M. D. Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain. *J. Physiology* **2018** 596.23pp 5723-5756.
- Scheiber, I. F.; Dringen, R. Astrocyte functions in the copper homeostasis of the brain. *Neurochemistry International*, **2012**, *2*, 556 – 565.
- Scheiber, I.F. Mercer, F.B. Dringen R. Metabolism and functions of copper in the brain. *Progress in Neurobiology* **2014**, *116*, 37- 57.
- Tiffany-Castiglioni, E.; Hong, S.; Qian, Y. Copper handling by astrocytes: Insights into neurodegenerative diseases. *Int. J. Devl. Neuroscience*, **2011**, *29*, 811-818.
- Zheng, W. Neurotoxicology of the brain barrier system: new implications. *Clinical Toxicology*, **2001**, *39* (7), 711-719.
- Zheng, W.; Aschner, M.; Gersai-Egea, J. Brain barrier systems: A new frontier in metal neurotoxicological research. *Toxicol. Appl. Pharmacol.* **2003**, *192*, 1-11.
- Zheng, W.; Monnot, A. D. Regulation of brain iron and copper homeostasis by brain barrier systems: implications in neurodegenerative diseases. *Pharmacology & Therapeutics* **2012**, *133*, 177-188.



Fig. 2. North Ronaldsay sheep cerebellum (adult)

Persistence of strong (neonatal) CTR1 expression in capillary endothelium of BBB. (copyright Elsevier Ltd)

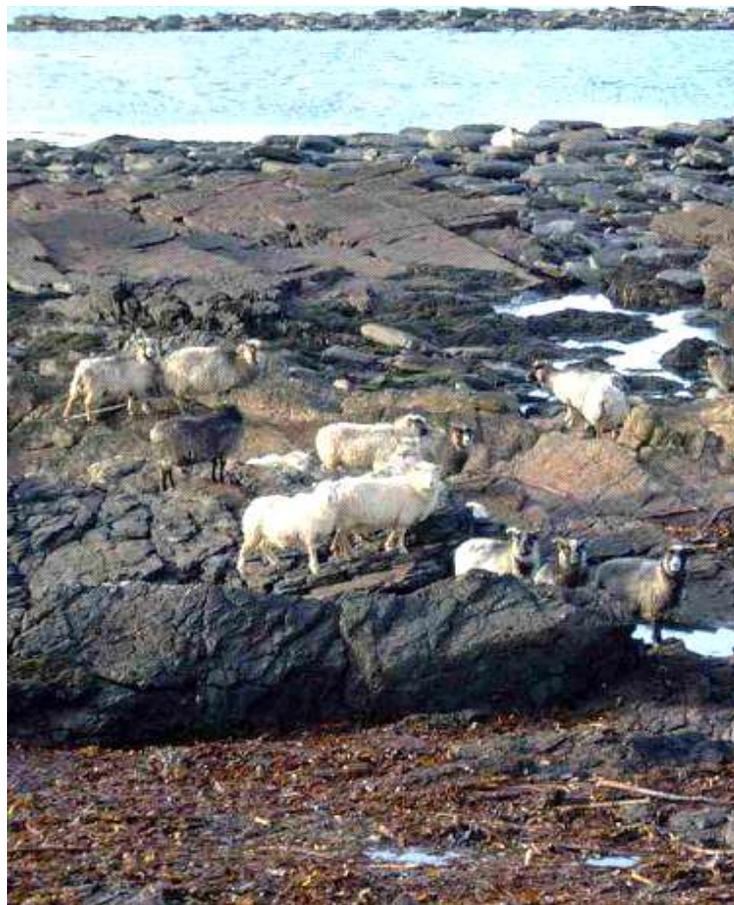


Fig 1. North Ronaldsay sheep on island foreshore

NR sheep have adapted to survive on seaweed which is very low (< 6ppm) in copper).