

Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration

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Abstract. The etiology of many neurodegenerative diseases has been only partly attributed to acquired traits, suggesting environmental factors may also contribute. Metal dyshomeostasis causes or has been implicated in many neurodegenerative diseases. Metal flux across the blood-brain barrier (the primary route of brain metal uptake) and the choroid plexuses as well as sensory nerve metal uptake from the nasal cavity are reviewed. Transporters that have been described at the blood-brain barrier are listed to illustrate the extensive possibilities for moving substances into and out of the brain. The controversial role of aluminum in Alzheimer's disease, evidence suggesting brain aluminum uptake by transferrin-receptor mediated endocytosis and of aluminum citrate by system Xc⁻ and an organic anion transporter, and results suggesting transporter-mediated aluminum brain efflux are reviewed. The ability of manganese to produce a parkinsonism-like syndrome, evidence suggesting manganese uptake by transferrin- and non-transferrin-dependent mechanisms which may include store-operated calcium channels, and the lack of transporter-mediated manganese brain efflux, are discussed. The evidence for transferrin-dependent and independent mechanisms of brain iron uptake is presented. The copper transporters, ATP7A and ATP7B, and their roles in Menkes and Wilson's diseases, are summarized. Brain zinc uptake is facilitated by L- and D-histidine, but a transporter, if involved, has not been identified. Brain lead uptake may involve a non-energy-dependent process, store-operated calcium channels, and/or an ATP-dependent calcium pump. Methyl mercury can form a complex with L-cysteine that mimics methionine, enabling its transport by the L system. The putative roles of zinc transporters, ZnT and Zip, in regulating brain zinc are discussed. Although brain uptake mechanisms for some metals have been identified, metal efflux from the brain has received little attention, preventing integration of all processes that contribute to brain metal concentrations.

Keywords: Aluminum, blood-brain barrier, brain efflux, brain influx, choroid plexus, iron, lead, manganese, mercury, zinc

Abbreviations

A β	amyloid- β
ACM	astrocyte-conditioned media
AD	Alzheimer's disease
Al	aluminum

ALS	amyotrophic lateral sclerosis
BBB	blood-brain barrier
BBr	brain/blood ratio
BCECs	brain capillary endothelial cells
CNS	central nervous system
CP	choroid plexus
CSF	cerebrospinal fluid
Cu	copper
ECF	extra-cellular fluid

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FCCP	carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazine
Fe	iron
Hg	mercury
K_{in}	influx transfer coefficient
Mn	manganese
Pb	lead
PD	Parkinson's disease
TfR-ME	transferrin-receptor mediated endocytosis
Zn	zinc

1. Metals in neurodegenerative diseases

Neurodegenerative diseases are characterized by activated inflammatory processes and the accumulation of protein deposits within brain parenchyma which lead to neuronal damage and loss. There is often progressive brain degeneration long before the development of a cascade of symptoms over 2 to 20 years that leads to increasing disability, ultimately contributing to death. The dominant risk factor of neurodegenerative diseases is age. There is an age-related increase of aluminum (Al), copper (Cu), iron (Fe), and zinc (Zn), but not manganese (Mn), in the brain [1–5].

The most common neurodegenerative disease is Alzheimer's disease (AD). It has a prevalence of < 5% at age 60, doubling every 5 years to > 20% at age 80 [6]. To date, only a small percentage of cases has been linked to acquired (genetic) traits [7–9]. These are characterized by aberrant processing and deposition of amyloid β protein precursor, leading to the formation of neurotoxic amyloid- β ($A\beta$) peptides and an aggregated insoluble polymer of $A\beta$ that forms the senile plaque [10]. Environmental and dyshomeostasis mechanisms involving metals have been suggested to contribute to the sporadic forms of AD. Among the metals most commonly suggested to contribute to AD are Al, Cu, Fe, and Zn [11–15].

The second most common neurodegenerative disease is Parkinson's disease (PD), and the similar syndrome parkinsonism. There are ~ 500,000 people in the United States who are believed to suffer from PD. The prevalence and incidence increase with age. Autopsy evidence of Lewy bodies, a hallmark of PD, was seen in ~ 16% of those over 80 years old [16]. This is a heterogeneous disease with a significant genetic component. Over 10 mutations have been linked to PD, and some identified, which account for 10 to 20% of cases. These include *Parkin* (PARK 2) mutations, which are

the most common single gene cause of PD, involved in 10 to 20% of PD cases. Other families of genetic mutations involve α -synuclein (PARK 1), PTEN-induced kinase (PINK 1; PARK 6), the protein DJ-1 (PARK 7), and a ubiquitin carboxy-terminal hydrolase [17–21]. As acquired traits have not been identified in the majority of the cases it has been suggested that this is likely to be an environmental disease [22]. Elevated Fe and Cu deficiency have been found in the substantia nigra in PD [23,24]. Occupational exposure to Al, Cu, Fe, lead (Pb), Mn, mercury (Hg) and/or Zn appear to be risk factors for PD, according to some epidemiological studies [22,25,26].

Wilson's Disease is less common, with a prevalence of 1/30,000 to 1/60,000, ~ 0.0015 to 0.003% [27–29]. It is the result of the autosomal recessive inheritance of a gene mutation for ATP7B [29]. Many mutations of this transporter have been reported. ATP7B encodes a P-type ATPase that specifically transports Cu. It is localized to the trans-Golgi network [30]. Functionally, it incorporates Cu into apo-ceruloplasmin and is important in the vesicular pathway of hepatic Cu transport into bile [31]. Mutation of ATP7B in Wilson's Disease leads to decreased biliary Cu excretion into bile, Cu accumulation in the liver and brain and the problems associated with excess Cu, including neurological and psychiatric abnormalities [30,32].

Menkes disease is an X-linked recessive disorder of Cu deficiency with an incidence of 1/100,000 births and a prevalence of ~ 1/250,000 to 1/350,000, ~ 0.0003 to 0.0004% [30,33,34]. The Menkes protein (MNKP) is also a Cu-translocating P-type ATPase, specific for Cu(I) [35], ATP7A, which has ~ 60% sequence homology and similar activity and intracellular localization as ATP7B [31]. ATP7A is involved in Cu delivery to secretory pathway cuproenzymes [36]. Mutation of ATP7A produces a different disease than ATP7B mutation due to the different expression patterns of these transporters [37]. Due to the disruption of Cu transport, resulting in reduced transport of dietary Cu across the basolateral membrane of enterocytes into hepatic portal circulation and across the BBB, Menkes disease is associated with cellular Cu deficiency, including very low brain Cu, leading to neuronal degeneration in infants [30,38].

Amyotrophic lateral sclerosis (ALS) (a.k.a.: Lou Gehrig's disease) is the most common motor neuron disease. It affects both upper and lower motor neurons. It has a prevalence of 1/20,000 to 1/80,000, 0.001 to 0.005% [39–41]. Autosomal dominant familial forms due to a mutation of Cu, Zn-superoxide dismutase

(SOD1) have been identified which account for about 20% of the cases [42]. The cause(s) and contributor(s) of sporadic forms are unknown. It has been suggested that elevated Al, Cu, Pb, Mn and/or Hg and low calcium (Ca) and magnesium contribute to ALS [43–45].

Huntington's disease has a prevalence of 1/10,000 to 1/40,000, ~ 0.0025 to 0.01% [46,47], which is considerably higher in some local regions [48,49]. It is an autosomal dominant inherited disease attributed to the increased production of a protein, huntingtin, which has an unknown function [50]. The disease is caused by CAG triplet repeat expansion coding for a polyglutamate sequence in the huntingtin protein. The extent of contribution of environmental factors to Huntington's disease has not been well determined. Elevations of brain Cu and Fe have been seen [23]. They may contribute to this disease [51].

Friedreich's ataxia has a prevalence of 1 to 4/100,000, ~ 0.001 to 0.004% [52,53]. More than 98% of cases have defects in transcription of frataxin, resulting in insufficient production of this mitochondrial Fe chaperone protein that is required for mitochondrial Fe homeostasis, maintenance of Fe-containing enzymes and protection from oxidative stress [54].

In summary, metal dyshomeostasis has been shown to cause or has been implicated in most of the most prevalent neurodegenerative diseases. Aluminum has been implicated in AD, PD and ALS. Copper has been implicated in AD, PD and ALS. It accumulates, due to mutation of ATP7B, and contributes to the toxicity of Wilson's disease. Conversely, Cu deficiency, due to mutation of ATP7A, contributes to the toxicity of Menkes disease. Iron has been implicated in AD, PD and Huntington's disease. Nearly all cases of Friedreich's ataxia are due to a transcription defect of an Fe chaperone protein. Hg and methyl Hg (MeHg) have been implicated in PD and ALS. Lead has been implicated in PD and ALS. Mn produces a syndrome, mannanism, similar to but clearly distinct from parkinsonism, creating concern that it might contribute to PD. This concern has been supported by some, but not by other, epidemiology studies. It has also been implicated in ALS. Zn has been implicated in AD and PD.

2. How metals might enter the brain

To produce neurodegeneration, it is assumed that the neurotoxic metal enters the central nervous system (CNS). Many metals that have been suggested to con-

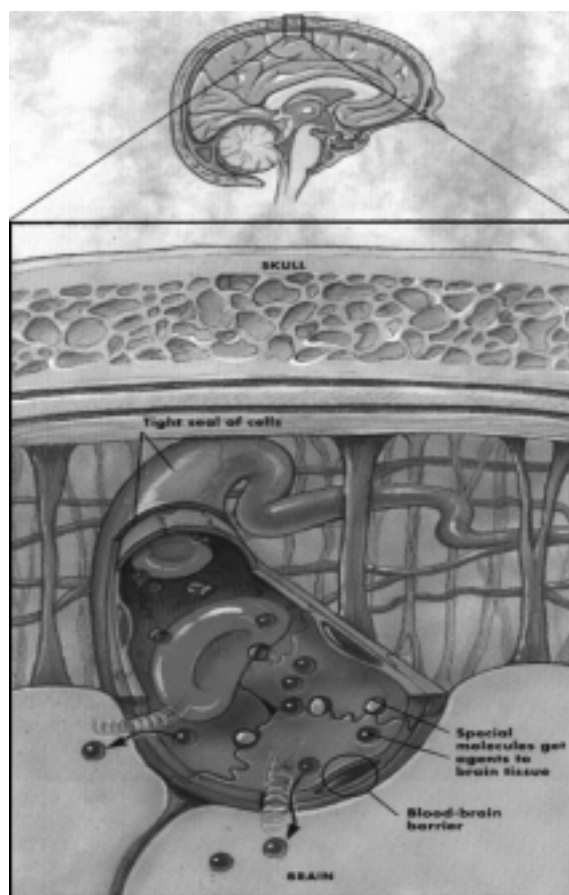


Fig. 1. Diagram of a cross-section of the human head and an expanded image showing a microvessel that contributes to the BBB and is the source of rapid substance exchange between blood and brain. The BBB is shown by the tight seals (junctions) between the endothelial cells, which greatly limit diffusion through the paracellular pathway between these cells. Two additional routes of flux across the BBB are shown for 1) substances that are sufficiently lipophilic to diffuse through the plasma membranes surrounding the lumen of the microvessel, and 2) substrates that can be transported across the endothelial cell membranes by special molecules (transporters). Reprinted with permission from Lydia Kibiuk [282].

tribute to neurodegenerative diseases are considered to be essential for human health in trace amounts (Cu, Fe, Mn and Zn). For Pb and tin there is circumstantial evidence for essentiality that is not yet convincing [55,56]. There is no good evidence for essentiality in the mammal for Al or Hg. Because metal species, such as the free metal ion and complexes of the metal with an amino acid or protein, such as transferrin, are quite hydrophilic, they would not be expected to be able to distribute across the blood-brain barrier (BBB) at a rate that is sufficient to meet the requirements of the brain. This is due to properties of the BBB that greatly limit diffusion of non-lipophilic substances into

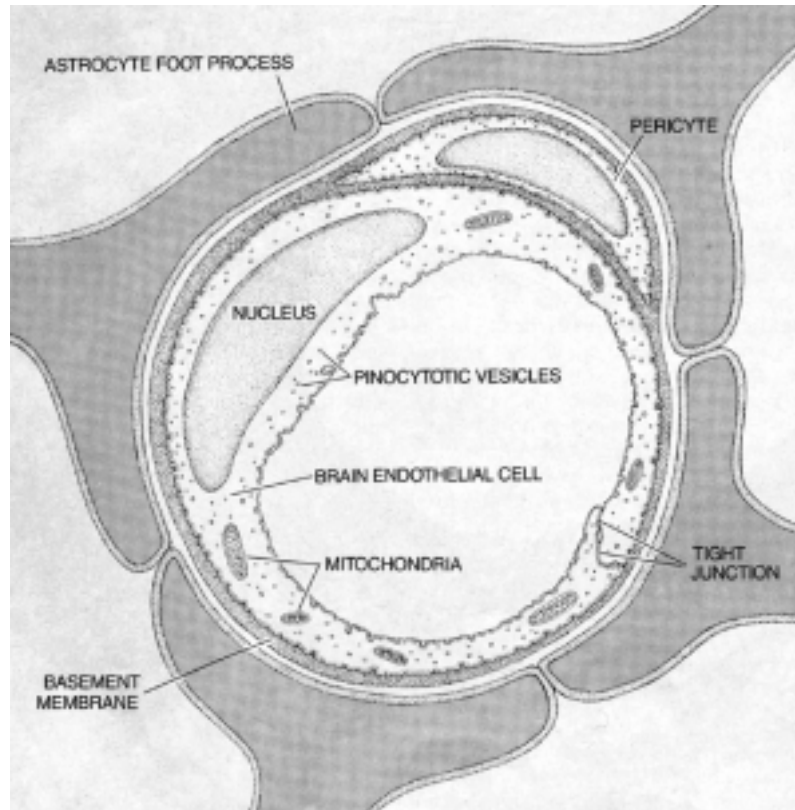


Fig. 2. Diagram of a cross section of a microvessel in the brain surrounded by an endothelial cell, a pericyte and astrocyte foot processes. Absent are fenestrations and much larger spaces between cells that are features of microvessels in other tissues that enable more exchange between blood and tissue. Reprinted with permission from Patricia J. Wynne [283].

and out of the brain [57]. As brain glucose demand greatly exceeds the rate of glucose diffusion across the BBB, Glut-1 mediates brain glucose uptake to meet the brain's needs. Similarly, the L (for leucine) system (a.k.a.: large neutral amino acid system, LAT1) mediates the bidirectional transport of leucine and phenylalanine across the BBB to provide the brain the amino acids it requires and remove excess amino acids and similar substances when they accumulate as metabolic products. Therefore, it is anticipated that metal distribution across the BBB might be transporter mediated. Metals are most often absorbed from the gastrointestinal tract, across the lungs, or through the skin. They then enter systemic circulation. The metal could then enter the CNS from the blood across the BBB or from the blood by crossing the choroid plexus (CP) into the cerebrospinal fluid (CSF), from which it can diffuse into the CNS. Alternatively, there is evidence some metals can be taken up by exposed sensory nerves in the nasal cavity and possibly enter the brain, as discussed below.

3. Mechanisms of metal flux across membranes

The mechanisms of distribution of substances across cell membranes include diffusion and carrier mediated transport [57,58]. Flux of substances between cells is primarily by paracellular diffusion. Brain entry through the intact BBB might be achieved by the very limited diffusion through the paracellular pathway. Diffusion through endothelial cell membranes is generally limited to small (usually < 700 dalton), lipophilic substances. There are exceptions. Elemental Hg is readily absorbed from the lung and diffuses across the BBB. Organomercurials such as methyl and dimethyl Hg are absorbed from the lung, gastrointestinal tract and via the percutaneous route and readily distribute across the BBB. Carrier mediated transport includes equilibrative, and energy-dependent transporters that are able to move substrates unidirectionally and against a concentration gradient, and receptor-mediated mechanisms which may operate by facilitated diffusion and are often bidirectional.



Fig. 3. Human cerebellar cortical blood vessels, shown to emphasize the small distance between any cell in the brain and a blood vessel comprising the blood-brain barrier. The scale bar = 40 μm . Reprinted from Brain Research Bulletin, Vol. 11, Issue 4, H. Duvernoy, S. Delon and J. L. Vannson, The vascularization of the human cerebellar cortex, pp. 419–480, 1983, with permission from Elsevier.

4. Metal transporters

Membrane transporters are often quite substrate (in the context of this review: metal species) specific. For example: the divalent metal transporter (DMT1, DCT1, Nramp2) transports divalent metals, but not metals in other valence states. Another example of substrate specificity is the much greater binding affinity of transferrin for Fe^{+3} than Fe^{+2} ($\log K_1 \sim 22$ vs. ~ 7) [59]. As a result, transferrin-receptor mediated endocytosis (TfR-ME) for Fe^{+3} is much greater than for Fe^{+2} . Furthermore, the affinity of the transferrin receptor for halotransferrin (diferric transferrin) is considerably greater than for monoferric transferrin [60]. TfR-ME is also believed to play a role in the transport of other trivalent metals into the brain, such as Al and Mn. An example of metal species specific transport at the BBB involves MeHg, which forms a complex with L-cysteine that is transported into the brain, and perhaps into and out of astrocytes, as discussed below.

5. The blood-brain barrier and its transporters

The barrier properties of the BBB are due to several components, including tight junctions between opposing plasma membranes of the endothelial cells that surround the microvessels that perfuse the brain, which

greatly limit paracellular diffusion between these cells. Endothelial cells form a monolayer on the inner surface of all blood vessels. In the human brain the luminal surface area of these cells is $\sim 20 \text{ m}^2$, ~ 100 - to 1000 -fold greater than the surface area of the CP. These cells are connected by tight junctions (zonulae occludens) in the CNS, compared to much larger ($\sim 4 \text{ nm}$) gaps between such cells outside of the CNS. These endothelial cells have a near total absence of 1) fenestrations (openings) through which substances might diffuse, 2) fluid phase endocytosis (cytoplasmic pinocytotic vesicles that might engulf substances outside of the cell and internalize them) and 3) receptor-mediated endocytosis. An exception to the latter is the specific process of TfR-ME. Surrounding the abluminal side of the brain endothelial cells are three more barriers; a basement membrane, pericytes that surround $\sim 30\%$ of the endothelial cell surface and astrocyte foot processes that cover $> 90\%$ of the surface of the endothelial cells and pericytes, as shown in Figs 1 and 2. These provide further lipid membrane barriers to diffusion of hydrophilic molecules. Substances that cross the BBB from blood enter brain extra-cellular fluid (ECF). Owing to the extensive distribution of the microvessels throughout the brain, as shown in Fig. 3, the greatest distance between a brain cell and a microvessel that comprises the BBB, and is the site of influx into or efflux from the brain, is ~ 30 to $50 \mu\text{m}$ (Arnold Scheibel, personal communication). This provides the opportunity for very rapid exchange of substances between blood and brain cells.

There is evidence that many transporters are expressed at the BBB. Some of these provide a functional barrier to influx at the BBB, such as P-gp, whereas others enhance brain uptake of substances from the blood. Many of the transporters that have been described at the BBB are shown in Table 1. The identity, localization and activity of some transporters have been well described; for others there has only been a functional description. It is anticipated that further transporters at the BBB will be identified as all of the functions of genes expressed by the brain endothelial cells are characterized. Transporters at the BBB mediate brain influx and/or efflux of some metals, as discussed below, and may provide a mechanism for metal removal from the brain to treat neurodegenerative disorders [61].

6. The choroid plexus and its transporters

The choroid plexuses are capillary networks in the two lateral, the third and the fourth ventricles of the

Table 1

Transporters at the BBB. Transporters, official symbol, location (L = luminal, A = abluminal), type (AT = active transport, FD = facilitated diffusion, RM = receptor mediated, SAT = secondary active transport), and typical substrates. Official symbols are upper case for human and lower case for non-human, typically mouse and rat. From Entrez Gene (<http://www.ncbi.nih.gov/entrez/query.fcgi?db=gene>), the UCSD Transport Classification Database (http://www.tcdb.org/hgnc_explore.php), the references cited which provide more details and citations to the primary literature, and other sources

Transporter	Official symbol	Location		Influx or efflux from brain	Type	Typical substrates	Ref
		L	A				
Nutrient transporters							
hexose (GLUT-1)	Slc2a1, SLC2A1	x	< x	I & E	FD	glucose, morphine-6- β -D-glucuronide	[58,260,261,262]
monocarboxylate (MCT1)	Slc16a1	x	x	I & E	FD	benzoic, lactic, lovastatin & pyruvic acids	[58,261]
anion exchange (band 3)	Slc4a, CL4A			I & E	FD	Cl ⁻ /HCO ₃ ⁻ , lactate, metal-anion complexes	[261]
large neutral amino acid (system L) (LAT1 & LAT2)	SLC7A5 (LAT1), SLC7A8 (LAT2)	x >	x	I	FD	branched or aromatic side chain amino acids; L-DOPA, α -methyl-DOPA, gabapentin, L-leucine, melphalan, L-phenylalanine, L-tryptophan, L-tyrosine	[58,261,263]
Na ⁺ -LNAA neutral amino acid			x	E		alanine, glycine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, valine	[264,265]
small neutral amino acid (system A)		x	< x	E	SAT	L-alanine, L-asparagine, L-cysteine, glutamine, glycine, L-histidine, L-proline, L-serine	[261,264]
neutral amino acid (system ASC) (ASCT1 & ASCT2)			x	E		L-alanine, L-aspartate, L-cysteine, L-glutamate, glycine, L-isoleucine, L-leucine, L-methionine, L-serine, L-threonine, L-valine	[261,264,266]
system T						thyroid hormones T ₃ & T ₄	[261]
system B ⁰⁺	SLC6A19		x	E		basic and neutral amino acids	[261]
system β						β -alanine, taurine	[261]
basic amino acid			x			lysine	[58]
acidic amino acid (system x ⁻)				I		L-aspartate, L-glutamate	[58,261]
system X ⁻			x	E		L-aspartate, L-glutamate	[261]
N-system		x	x		FD	L-asparagine, glutamine, glutamate, L-histidine, L-serine	[264]
excitatory amino acid (EAAT1-3)	SLC1A family		x			aspartate, glutamate	[261]
amine (cation, system y ⁺)	Slc7a, SLC7A families	x	< x		I	L-arginine, choline, L-cysteine, lysine, ornithine	[58,261]
β -amino acid		x	x			taurine	[58]
saturated fatty acids						octanoate	[58]
urea	Slc14a, SLC14A families				FD		
purine			x			adenine	[58]

Table 1, continued

Transporter	Official symbol	Location		Influx or efflux from brain	Type	Typical substrates	Ref
		L	A				
Neurotransmitter transporters							
norepinephrine	Slc6a2, SLC6A2			I		norepinephrine	[261]
serotonin	Slc6a4, SLC6A4			I		serotonin	[261]
GABA (GAT2, BGT1)	Slc6a, SLC6A families			I		GABA	[261]
Nucleoside transporters							
equilibrative nucleoside transporters (ENTes & ENTei)	Slc29a, SLC29A families	x	x	I & E		purine and pyrimidine nucleosides	[58,260,261,263]
concentrative nucleoside transporters (Cnt2, Cnt3, N2, N3)	Slc28a, SLC28A families	x	x	I		purine and pyrimidine nucleosides	[57,260,261,263]
Ion transporters							
Na/K-ATPase	atp1, ATP1 families	x	< x	K into and Na out of BCEC	AT	potassium	[260,261]
Ca-ATPase	Atp2c2	x	x		AT	calcium	[261]
sodium		x			AT	sodium	[58]
potassium, chloride	Slc12a, SLC12A families		x		AT	potassium/chloride cotransporter	[58]
Metal transporters							
divalent metal (DMT1, DCT1, Nramp2)	Slc11a2, SLC11A2					divalent Ca, Fe, Mn, Cd, Cu, Ni & Pb; Zn?	[263,267]
ferroportin	Slc11a3		x	I		divalent metals	[268]
ZIP6 (LIV-1)	SLC39A6					Zn	[238]
Menkes disease Cu-transporting ATPase	Atp7a			E		Cu	[24,36]
ttr1	SLC31A1			I			[224]
ATP-binding cassette (ABC) transporters							
P-glycoprotein (P-gp) multidrug resistance transporter (mouse Mdr1a [Mdr3], Mdr1b [Mdr1], & Mdr2; human MDR1 & 2)	Abcb, ABCB families			E	AT	organic, hydrophobic, amphipathic cations: β -adrenergic blockers, anthracyclines, cyclosporine A, digoxin, etoposide, glucocorticoids, loperamide, morphine, phenytoin, vecuronium, verapamil, vinca alkaloids	[260,261,263, 269,270]
multidrug resistance-associated protein (MRP1)	Abcc1, ABCC1	x		E	AT	amphipathic anions & glutathione, glucuronide & sulfate conjugates: benzylpenicillin, doxorubicin, etoposide, methotrexate, vinblastine, vincristine	[57,261]

Table 1, continued

Transporter	Official symbol	Location		Influx or efflux from brain	Type	Typical substrates	Ref
		L	A				
MRP2 (c-MOAT)	Abcc2, ABCC2			E	AT	organic anions	[271]
MRP3 (MOAT-D)	Abcc3, ABCC3	x		E	AT	methotrexate, anionic conjugates	[260,261,263]
MRP4 (MOATB)	Abcc4, ABCC4	x		E	AT	nucleoside-based antivirals	[260,261,263,269]
MRP5 (MOAT-C)	Abcc5, ABCC5	x		E	AT	cyclic nucleotides, glutathione conjugates, organic anions	[260,261,263,269]
MRP6 (MOAT-E)	Abcc6, ABCC6	x		E	AT	peptides	[260,261,263,269]
breast cancer resistance protein (BCRP)	Abcg2, ABCG2	x		E	AT	similar to P-gp substrates; camptothecin derivatives, daunorubicin, doxorubicin, mitoxantrone, topotecan	[261,272]
Organic cation transporters							
OCT1	Slc22a1, SLC22A1			I & E		monoamine neurotransmitters, cationic neurotoxins	[261]
OCT2	Slc22a2, SLC22A2			I & E		monoamine neurotransmitters, amantadine, memantine	[260,261]
OCT3	Slc22a3, Slc22a8, SLC22A3			I & E		amphetamine, desipramine, dopamine, serotonin	[261]
OCTN1	Slc22a4, SLC22A4			I & E		antiarrhythmic drugs, tetraethylammonium verapamil	[261]
OCTN2	Slc22a5, SLC22A5			I & E		β -lactam antibiotics, L-carnitine, tetraethylammonium	[261]
Organic anion transporters							
organic anion transporting polypeptide 1 (oatp1, OATP1)	Slco1a1, Slc21a1, SLC04A1	x	x	I & E		bulky organic cations, glucuronide conjugates, steroid hormones, estrone sulfate, indinavir, nelfinavir, opioid agonists, ouabain	[261,269]
Oatp2, OATP2	Slco1 family, SLC01B1, SLC21A6	x	x	I & E		amphipathic anions: bile acids, cholate, digoxin, estrogen conjugates, opioid agonists, ouabain, taurocholate; dehydroepiandrosterone sulfate and estrone-1-sulfate efflux; [26]-enkephalin; 3,5,3-triiodo-L-thyronine	[260,261,263,269]
Oatp3	Slco1a2, Slco1a5, SLC21A6			I & E		digoxin, [26]-enkephalin, fexofenadine, 3,5,3-triiodo-L-thyronine	[261]
BBB-specific anion transporter type 1 (Bsat1, oatp14)	Slco1c1, Slc21a14	x		I & E		T ₄	[273]
OATP-A	SLC01A2			I & E?		amphipathic anions, bile acids, deltorphin II, estrone-1-sulfate, fexofenadine, opioid peptides	[260,261,263,269]
OAT1	Slc22a6, SLC22A6			E		small, hydrophilic organic anions: AZT, benzylpenicillin, methotrexate, NSAIDs, PAH	[261]
OAT3	Slc22a8, SLC22A8		x	E		benzylpenicillin, cimetidine, dehydroepiandrosterone sulfate, estrone sulfate, indoxyl sulfate, PAH	[263]

Table 1, continued

Transporter	Official symbol	Location		Influx or efflux from brain	Type	Typical substrates	Ref
		L	A				
Arachidonic acid derivative transport							
Anandamide membrane transporter		x	x			anandamide	[274]
Receptor-mediated peptide transport							
transferrin receptor	Tfrc, TFRC	x	x	I	RM	transferrin-metal (Al, Fe, Mn) complexes	[58,263,275]
melanotransferrin (P97) receptor				I		Fe	[263]
insulin receptor and insulin-like growth factor receptor (IGF-R)	Insr, INSR	x	x		RM	insulin receptor and insulin-like growth factor	[57]
leptin receptor (OBR)	Igf1r, IGF1R Lepr, LEPR						[57]
neonatal Fc receptor (FcRN)	Fcgrt, FCGRT		x	E		immunoglobulin G	[57]
scavenger receptors (SR-AI [SCARA1] & SR-BI)	MSR1, Scarb1, SCARB1	x		I		amyloid- β peptide, apolipoprotein A-I, cholesterol esters	[57,263]

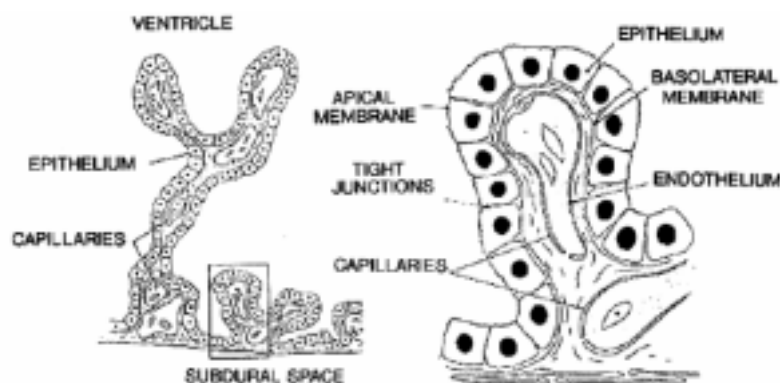


Fig. 4. Left: A diagram of a choroid plexus showing its extension into a ventricle in the brain. Right: Enlargement of the box in the left panel showing the tight junctions between the epithelial cells that comprise the barrier between capillary contents and the cerebrospinal fluid in the ventricle surrounding the choroid plexus. Reprinted with the permission of John Wiley & Sons, Inc [284].

brain. They are surrounded by a monolayer of epithelial cells that have tight junctions (Fig. 4). They are highly vascular and are the sites of CSF production. Substances that cross the CP from blood enter the CSF. Influx transfer coefficients (K_{in}) are generally greater in the CP than across the BBB into the brain of the intact animal (Tables 2 and 3). However, the CP sequesters many metals [62]. This results in low metal flux into the CSF (Table 4). The CP has been less studied than the BBB. There is evidence for the expression at the CP at higher levels than in the liver, kidney or ileum of Mrp 1, Mrp 4, Mrp 5, oatp3, Menke's transporter,

DMT1, equilibrative nucleoside transporter (Ent) 1 and peptide transporter (Pept) 2 [63]. Additionally, expression of organic cation transporters (Oct) N1 and N2, oatp2, organic anion transporters (oat) 2 and 3 and concentrative nucleoside transporters (Cnt) 1 and 2 was detectable in the CP, but was lower than in the liver, kidney or ileum. Expression of Mrp 2 and 3, Oct1, Oct2, Oatp1, Oatp4, Oatp5, Oatp12, Oat-K, sodium taurocholate co-transporting polypeptide (Ntcp), bile salt export protein (Bsep), ilial bile acid transporter (Ibat), Mdr1a, Mdr1b, Mdr2, Oat1, Ent2, Pept1, and ATP-binding cassette (Abc) G5 and G8 was very low [63].

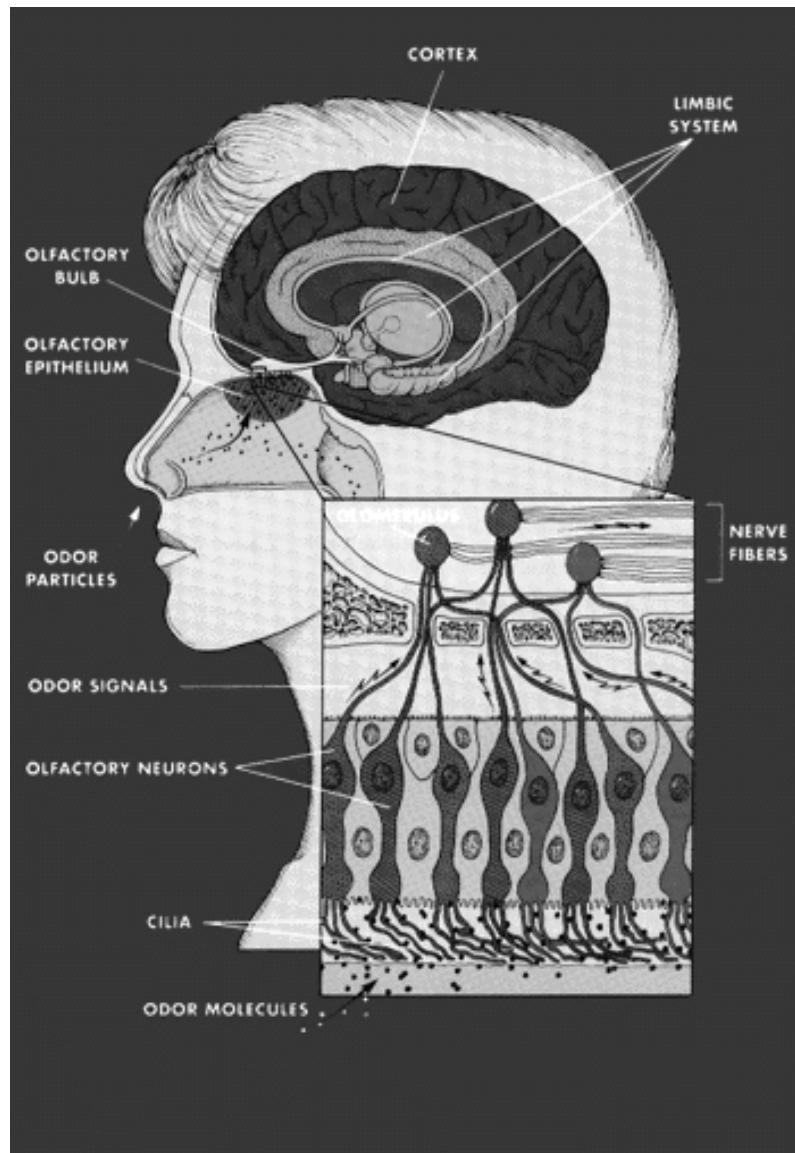


Fig. 5. Diagram showing the olfactory epithelium on the roof of the nasal cavity and, in the insert diagram, how molecules can be taken up by the cilia of the olfactory neurons and distribute to the olfactory bulb. For substances, such as Mn, which can distribute trans-synaptically to connecting neurons, the diagram emphasizes the neural connections to limbic system components. Reprinted with permission from Lydia Kibiuk [285].

Expression of ZnT1, ZnT3, ZnT4 and ZnT6 has been reported in the choroid plexus epithelial cells that comprise the barrier between blood and CSF [64], but not the BBB.

7. Brain metal entry via the olfactory and trigeminal neurons

In addition to the routes of distribution from blood to brain through the BBB and CP, it has been known for

some time that proteins (viruses) can distribute from the nasal cavity into the olfactory neuron, the only site where the CNS is exposed to the environment (Fig. 5), and then trans-synaptically beyond the olfactory neuron into other brain regions [65]. Trans-synaptic movement is involved in the distribution of tetanus virus from muscle to spinal cord ganglia, the site of its toxicity. It is known from studies in rats and pike fish that Mn, and perhaps nickel (Ni), can enter the brain directly by this route [66–72]. Studies in mice suggested rubidium and

thallium may enter the brain by olfactory transport [73, 74]. There is some disagreement on the extent of this mechanism of brain entry. It appears that this is not the major route of brain entry of metals. Other metals, such as cadmium, cobalt, Fe, inorganic Hg and Zn enter the olfactory bulb from the nasal cavity but their distribution beyond that into other brain regions in the fish or rat is much less or not detectible [67,71,75–79]. However, there are results in fish suggesting some distribution of inorganic Hg and tributyl tin into the brain by routes other than through the BBB, which were suggested to be uptake by water-exposed sensory nerves [80,81].

For Al, the studies assessing its ability to enter the brain from the nasal cavity are more controversial. The presence of Al and the development of neuropathology following intranasal Al lactate application were seen in the olfactory bulb, pyriform cortex, hippocampus and cerebral cortex, but not in cerebellum, brainstem or spinal cord [82]. The Al lactate was introduced as a 10% solution in GelFoam® which was placed in the upper nasal cavity for 1 month. Concerns about this experimental approach include the possibility that this prolonged exposure resulted in mechanical disruption of the olfactory epithelia [83]. In a meeting abstract this group also reported rabbit brain Al uptake from a 75 mM suspension of a lipophilic Al flavonol complex following its intranasal administration [84]. Granulomas, containing Al and auto-fluorescence, which were attributed to the Al flavonol, were seen in the pyriform cortex, basal forebrain, hippocampus and neocortex. The possibility that Al can enter the brain from the nasal cavity following nose-only inhalation exposure to Al chlorohydrate was assessed in rats [85]. The rats had significantly greater Al concentration in the olfactory bulb than in non-olfactory brain regions. Rats exposed to lipophilic Al acetylacetonate under conditions designed to maximize inhalation via the nasal-olfactory system had elevated brain Al [86]. Although Al was seen in the olfactory bulb, cortex, hippocampus, and entorhinal area, it was also seen in the cerebellum, which is not within, or directly connected neuronally to, the olfactory pathway. The Al may have entered the cerebellum from the CSF, rather than through neurons.

In addition to the potential for brain uptake from the nasal cavity by the olfactory nerve, the uptake of Mn by the sensory branch of the trigeminal nerve within the nasal epithelium [87] has been described in rats and mice given nasal route-only Mn chloride exposure [88]. The Mn appeared in the trigeminal ganglia and spinal trigeminal nucleus. The extent of Mn uptake by this route and its distribution to other brain regions has not been reported.

8. The role of transition metal-induced oxidative injury in neurodegenerative diseases

The transition metals Fe and Cu as free ions provide oxidation/reduction chemistry that can produce free radicals and reactive oxygen species. This has been implicated in the oxidative injury seen in some neurodegenerative diseases, particularly AD. Elevated Fe, Cu and Zn have been seen in the senile plaques of AD. This might contribute to neurodegenerative diseases through increased oxidative injury, A β aggregation and/or other mechanisms [89–91].

9. Aluminum and Alzheimer's disease

There is considerable published evidence supporting as well as not supporting a role for Al in AD. There have been numerous epidemiology studies that addressed the relationship between drinking water Al concentration and dementias, including AD [92,93]. A number of studies, particularly those with larger numbers of subjects, show a small, statistically significant, increased odds ratio associated with higher Al concentration in the drinking water. There are several concerns about this approach to assess the contribution of Al to AD. One is that the subjects were typically adults or the elderly yet most studies utilized single, or only a few, drinking water Al concentrations obtained within a short period of time prior to the study. This may not reflect Al exposure during the prior years of the subjects' lifetime. Another is that drinking water provides only 1 to 2% of the typical human's total Al exposure, although absorption of Al from drinking water is more complete than from food, the primary source of Al for most humans [94,95].

There are numerous reports of decreased performance on cognitive examinations in Al-exposed industrial workers, suggesting Al might contribute to neurodegeneration. Underground miners who were exposed by inhalation to finely ground Al and Al oxide "McIntyre Powder" performed cognitive state examinations less well than non-exposed subjects [96]. Slower psychomotor reaction was reported in workers who were occupationally exposed for several years to high Al concentrations [97]. Al smelting plant pot-room workers reported frequent loss of balance and memory loss. Memory tests showed impairment, and neurologic examination revealed signs of reduced coordination [98]. Adverse effects on short-term memory, learning, and attention were reported in ship-

yard Al welders who had evidence of Al accumulation [99]. Some evidence was reported in Al welders for decrements in tasks requiring working memory and time limited tasks involving visually presented material [100]. Al exposure-related increases in fatigue, mild depression, and memory and concentration problems; decrements in tasks demanding complex attention and the processing of information in the working memory system; and pathological electroencephalographic changes were seen in Al welders [101]. Adverse effects on reaction time, balance and color discrimination were observed in workers in Al re-melting, although the effects could not be definitively attributed to Al [102]. Differences in neurobehavioral tests were seen in workers exposed to Al, compared to controls [103].

On the other hand, there are numerous reports of studies suggesting Al does not induce cognitive loss in industrial workers. In an unmatched case control study of 198 AD cases and 340 controls, no evidence was found to support an association between having worked in an Al factory and increased risk of AD [104]. No great increased risk of neurological disorders was observed in a cross sectional study of 63 current and former potroom workers with at least 10 years of Al exposure compared to a group of 37 controls [105]. No cognitive decline was observed among 32 workers in an Al powder plant who had significantly elevated plasma and urine Al concentrations, compared to the control group [106]. Furthermore, no adverse effects on the nervous system were observed in 33 potroom, 86 foundry, and 16 flake powder workers [107]. A non-significant association was found between AD and occupational Al exposure (odds ratio 1.46, 95% confidence interval 0.62 to 3.42) in 89 subjects diagnosed with probable AD, suggesting to the authors that occupational exposure to Al is not likely to be an important risk factor for this disease [108].

Another approach to address the role of Al in AD has been to determine if brain Al concentration is greater in victims of this disease than other subjects. Brain Al has been measured in bulk (typically 30 to 50 mg) brain samples. It was the observation that the average brain Al concentration in five biopsied or necropsied AD patients was a few-fold higher than in three normal brains [109] that ignited the controversy of the role of Al in AD. Due to the mixed results in ~ 15 studies, most of which were summarized in [92], this approach did not resolve the controversy. As it was hypothesized that the Al concentration associated with specific brain structures characteristic of AD may be

elevated, which might not be reflected in bulk brain samples, microprobe techniques were employed to determine the presence, and in some cases concentration, of Al in senile plaques and neurofibrillary tangles, the neuropathological endpoints indicative of AD. As with the results of bulk brain Al analyses, the results of the ~ 15 studies of Al in plaques and ~ 15 studies in neurofibrillary tangles were not consistent. Most of the studies were summarized in [92]. Some investigators have concluded that the higher Al concentrations found in some studies are the result of contamination. Others defend their observations of higher Al in AD brain, citing extensive procedures to avoid contamination, the use of unstained tissue and comparison of brain tissue from AD and control subjects that was identically processed [110]. However, even if Al accumulation does occur in AD, it does not prove that Al has a cause-effect relationship in the etiology of this disease.

Further suggestion that Al may play a role in the etiology of AD comes from the results of studies that showed Al can produce detrimental changes in the brain that are also seen in AD. Oxidative stress appears to be enhanced in AD. Al can increase oxidative stress that is induced by Fe and other metals [111–113]. Al has been shown to induce apoptosis, which is seen in AD [114–116]. Al has the ability to produce changes that result in an increase of neurofibrillary tangle-like endpoints [117–120]. However, in animal studies the Al-induced changes are different than seen in AD, as they do not have the paired helical filaments of the latter [121]. As with neurofibrillary tangle-like endpoints, Al has been reported to increase A β endpoints, including A β production [122], plaque deposition [123,124], β -pleated sheet formation [125–127], polymerization and aggregation of A β protein [128–130], acceleration (with oxidative stress) of an aberrant splicing isoform of the presenilin-2 gene [131] and inhibition of A β peptide catabolism [132]. However, many of these studies used Al concentrations above those that are physiologically relevant. More details and a hypothesis that integrates many of these endpoints implicating Al in AD was recently published, along with a compilation of many other effects of Al on the CNS [133]. Furthermore, it is unknown if an effect seen after short-term high-level Al exposure would occur with long-term (perhaps life long) Al exposure at lower levels, e.g., exposure conditions relevant to humans.

10. Brain aluminum influx and efflux

The comparative flux of Al through the BBB and a lateral ventricle CP was assessed by concurrent micro-

dialysis of the rat's frontal cortex, a lateral ventricle and a jugular vein after i.v. Al injection. The Al was injected as Al citrate, the predominant small molecular weight Al species in plasma, as a dose that exceeded the metal-binding capacity of transferrin, therefore favoring Al citrate as the Al species most likely to be circulating in blood and available to enter the brain. The Al concentration in dialysate exiting the cortex peaked in the first 5 minute sample and was higher than in dialysate exiting the lateral ventricle [134]. The rapid appearance of Al in the frontal cortex was attributed to brain Al entry through the BBB rather than the CP-CSF route. This was concluded from 1) the lower Al concentration in the CSF than in the frontal cortex, 2) because the bulk flow of brain ECF from brain parenchyma to lateral ventricles [135] would be expected to impede the movement of substances from CSF to brain ECF, and 3) because some frontal cortical regions in the rat are a considerable distance from CSF, 1.5 mm from the lateral ventricle and 1 mm from subarachnoid space. It was not thought that Al could diffuse from CSF to the frontal cortex within 5 minutes. Although Al rapidly enters both brain ECF and CSF from the blood, it appears that Al primarily enters the brain through the BBB. The ability of Al to distribute into the CSF was further demonstrated by the presence of measurable Al within 30 minutes after its i.v. injection as the chloride and sulfate [136,137].

It was suggested that Al is transported into the brain by TfR-ME. Al transferrin accounts for ~ 93% of Al in blood ECF (plasma) [138]. Transferrin receptor density in the forebrain of chronic hemodialysis patients correlated with Al concentration in that brain region, suggesting Al might enter the brain via TfR-ME [139]. Al transferrin did associate with rat brain synaptosomes, with a B_{max} comparable to and a K_D slightly lower than Fe transferrin. Al transferrin and Fe transferrin competed for transferrin binding, supporting the suggestion that Al might enter the brain via TfR-ME [140]. Four hours after the i.v. injection of ^{26}Al transferrin to rats, when blood had 0.08% of the dose/ml, 0.003% of the dose was found/g brain [141]. Assuming that 1) the distribution of the injected Al transferrin was originally confined to the vascular compartment, 2) that the Al transferrin was cleared from blood to brain over the four hours by a first order process, and 3) that the K_{in} of Al transferrin is comparable to the value for Fe transferrin (0.08×10^{-5} ml/sec/gm), TfR-ME could account for the observed brain influx of ^{26}Al .

However, the rapid brain entry of Al observed after i.v. Al citrate injection [134] cannot be explained

by TfR-ME. Assuming 1) transferrin saturation by Al, 2) comparable K_{in} values for Al and Fe by TfR-ME, and 3) the initial confinement of Al to brain ECF, then the maximum brain ECF Al concentration that could be achieved by TfR-ME in 20 minutes would be ~ 0.15 μM . This is very much lower than the brain ECF Al concentration seen in microdialysis studies (18–245 μM) [142], which was estimated from dialysate Al concentrations and 3.25% relative recovery of Al by microdialysis probes [143]. The marker of BBB integrity (4-trimethylammonium antipyrine) administered in this study showed no significant BBB disruption. The results suggested a mechanism for brain Al uptake other than TfR-ME. The observed reduction of brain Al uptake, introduced as "free" Al in bicarbonate buffer, in the presence of transferrin [144] supports the suggestion that non-transferrin bound Al is able to enter the brain by a more rapid mechanism than provided by TfR-ME. Further evidence for non-TfR-ME mechanism(s) of brain Al uptake was the lack of significant difference in brain Al uptake in hypotransferrinemic versus control mice and after treatment with a transferrin receptor antibody (R17 208), in mice infused with Al citrate [145].

The rate of brain Al appearance after i.v. Al citrate injection was too rapid to be explained by diffusion through the BBB. The rate of Al citrate flux through a membrane by diffusion was estimated to be 4×10^{-16} mol cm^{-2} sec^{-1} [146]. Brain capillary surface area was estimated to be 240 cm^2/g brain [147]. Brain ECF occupies ~ 15% of brain volume. Unbound Al in plasma after an i.v. Al injection was estimated to be ~ 1 mM based on an Al concentration of ~ 30 μM in the dialysate from microdialysis probes in the blood [134] and a relative recovery, using microdialysis, of Al from plasma ultrafiltrate of 3.25%. Therefore, if 4×10^{-16} mole of Al diffuses through 1 cm^2 of membrane in 1 second, ~ 3×10^{-11} mole of Al could diffuse through 240 cm^2 of capillary endothelial cells into 1 gram of brain in 5 minutes. If this distributed within brain ECF, the Al concentration would be ~ 2×10^{-17} M, producing a brain/blood ratio (BBr) of 0.0002. However, the BBr seen 5 minutes after i.v. injection of Al citrate was ~ 0.15 [134], suggesting brain Al citrate influx is transporter mediated.

Using gallium chloride as a substitute for Al, and the *in situ* brain perfusion method, the K_{in} was found to be 0.03 to 0.06×10^{-5} ml/sec/gm [148]. However, a number of studies have shown that gallium does not model Al well. The K_{in} for Al was reported for the free ion and in the presence of transferrin (at an Al:transferrin

Table 2
Influx transfer coefficients (K_{in}) into the brain for neurotoxic metals

Metal	Chemical species of the metal	Experimental subject	K_{in} Mean, as (10^{-5} ml/sec/gm)	Experimental method	Reference
Al	citrate	rat ^a	0.03 and 0.04	i.v. infusion	[149]
Al	Al ³⁺ ion partially associated with transferrin	Sprague Dawley rat	800 and 1340 200 and 400	[276]	[144]
Fe	Fe ³⁺	ICR mouse	0.195	bolus i.v. injection and graphical analysis [277]	[203]
Fe	chloride	Fischer 344 rat	0.03 to 0.04	bolus i.v. injection and graphical analysis [277]	[148]
Fe	with rat serum albumin	Wistar rat	0.1 to 1.5	bolus i.v. injection and graphical analysis [277]	[209,214]
Fe	chloride plus ascorbic acid	adult mouse ^a	0.14 to 0.2	i.v. infusion	[207]
	transferrin		0.08 to 0.1		
Fe	transferrin plus ascorbic acid	young mouse ^a	0.16 to 0.21		
Fe	chloride plus ascorbic acid	Wistar rat	0.14 to 0.2	i.v. infusion	[207]
Fe	no transferrin	rat ^a	0.06 to 0.10	i.v. infusion	[278]
Fe	transferrin	Sprague Dawley rat	13 to 27 8 to 28	modified Takasato	[221]
Pb	chloride	Wistar rat	1.8 to 2.3 for tracer ²⁰³ Pb concentration	i.v. infusion	[240,241]
Pb	acetate		~15-fold lower for ~30 μ M plasma [lead]		
Pb	chloride	Wistar rat	130 to 225 for perfusate lead 0.1 to 4 μ M	[276]	[243]
Mn	acetate tetrahydrate ion	Fischer 344 rat	0.3 to 48, for non-saturable uptake	i.v. infusion	[177]
Mn	transferrin in plasma	Wistar rat	13	bolus i.v. injection and graphical analysis [277]	[184]
	in saline		0.05		
Mn	in blood	Sprague Dawley rat	0.15	modified Takasato [279]	[180]
Mn	ion	Sprague Dawley rat	12 to 27 5 to 10	modified Takasato [280]	[181]
	citrate		5 to 13		
	transferrin		3 to 51		
Mn	ion	Wistar rat	2 to 13	modified Takasato [280]	[192]
	transferrin	Wistar rat	3		
		+/b Belgrade rat	5.3		
		b/b Belgrade rat	4.0		
		Wistar rat	8.4		
		+/b Belgrade rat	10.5		
		b/b Belgrade rat	5.3		
Hg	chloride	Wistar rat	0.7	i.v. infusion and graphical analysis [277]	[281]
Zn	chloride	Wistar rat	1.5 to 2.2	i.v. infusion	[226]
Zn	chloride	Wistar rat	0.9	bolus i.v. injection and graphical analysis [277]	[227,228]
Zn	albumin L-histidine	Wistar rat	28 to 44 332 to 458	modified Takasato [243]	[229]

^aStrain not reported.

molar ratio of 5:1) (Table 2). Owing to the 2 metal binding sites of transferrin, a maximum of 40% of the Al would be transferrin bound [144]. This K_{in} might be an underestimate if there was significant efflux of Al from the brain during the 45 second washout that followed brain perfusion. In contrast, a much lower value (Table 2) was reported in a second, also non-peer

reviewed, report, which was based on quite variable results after a 50 hour Al perfusion [149]. This may be a significant underestimate of K_{in} due to probable brain Al efflux during the 50 hours.

Rats given daily L-glutamate injections for two weeks prior to a single i.v. Al injection had higher brain Al concentrations than saline-injected rats [150].

Table 3
Influx transfer coefficients (K_{in}) into the choroid plexus for neurotoxic metals

Metal	Chemical species of the metal	Experimental subject	K_{in} Mean, as (10^{-5} ml/sec/gm)	Experimental method	Reference
Fe	no transferrin	Sprague Dawley rat	17	modified Takasato	[221]
	transferrin		22		
Pb	chloride	Wistar rat	48	i.v. infusion [276]	[240,241]
Pb	chloride	Wistar rat	1900 to 2500		
Mn	acetate tetrahydrate	Fischer 344 rat	285 to 2380	i.v. infusion [279]	[177]
Mn	in saline	Sprague Dawley rat	1772		
	in blood		727		[180]
Mn	ion	Sprague Dawley rat	850	modified Takasato [280]	[181]
	citrate		463		
	transferrin		1383		
Zn	chloride	Wistar rat	10	bolus i.v. injection and graphical analysis [277] [228]	

Table 4
Influx transfer coefficients (K_{in}) into CSF for neurotoxic metals

Metal	Chemical species of the metal	Experimental subject	K_{in} Mean, as (10^{-5} ml/sec/gm [ml])	Experimental method	Reference
Fe	chloride	Fischer 344 rat	0.012	bolus i.v. injection and graphical analysis [277]	[148]
Pb	chloride	Wistar rat	1.1		
Mn	acetate tetrahydrate	Fischer 344 rat	0.8 to 1.7	i.v. infusion	[177]
Zn	chloride	Wistar rat	0 to negligible	bolus i.v. injection and graphical analysis [277] [228]	

This finding was attributed to formation of an Al L-glutamate complex that crossed the BBB. The authors cited a log AlL-glutamate stability constant of 15.04 for the 1:1 Al:L-glutamate complex in support of their conclusion. However, a more accurate value is 7.69 [151]. Based on this lower stability constant and plasma and CSF glutamate concentrations of 58 and 26 μ M, respectively, it was concluded that glutamate is not competitive with most other ligands, such as citrate, for Al in physiological systems [151].

Addition of magnesium D-aspartate to the administration of Al L-glutamate was found to reduce brain Al accumulation [152]. Considering that D-aspartate serves as a substrate for glutamate transporters, it was suggested that it might cause the counter-transport of Al citrate. To characterize the transporter for Al citrate at the BBB, studies were conducted with RBEC1 cells, an immortalized rat brain capillary endothelial (a.k.a.: brain microvascular) cell line (rat BCECs) [153]. Al citrate uptake was temperature- and concentration-dependent, but not energy-dependent. The last conclusion was based on lack of significant inhibition of Al citrate uptake in the presence of azide (which inhibits mitochondrial cytochrome oxidase [complex IV] to prevent electron transfer to oxygen) and 2-deoxy-D-glucose (a glycolysis inhibitor). Uptake was Na dependent (assessed by replacement of sodium by choline) and concentrative. Al citrate uptake was inhibited by ligands for the Na-independent L-glutamate/L-cystine

exchanger system Xc^- (L-glutamate and L-cystine) but was increased by pre-loading the cells with these ligands. The latter results were interpreted as a *trans*-stimulatory effect. The authors concluded that system Xc^- is a potential candidate for Al citrate uptake into the brain across the BBB [153].

In conclusion, there appear to be at least two mechanisms by which Al may distribute from blood to brain across the BBB. They are TfR-ME and one or more rapid, transporter-mediated, process(es) that probably transfer(s) Al citrate.

Further assessment of Al distribution across the BBB was conducted by microdialysis during steady state Al concentrations, achieved by i.v. Al citrate infusion, over an 8-fold range of infusion rates [142]. As in bolus dose studies, the BBr was ~ 0.15 . When diffusion mediates transmembrane flux, $Cl_{in} \times C_{bl.u}$ should equal $Cl_{out} \times C_{br.u}$, where Cl_{in} is the clearance into the brain (influx), $C_{bl.u}$ is the unbound concentration in blood ECF, Cl_{out} is the clearance out of the brain (efflux), and $C_{br.u}$ is the unbound concentration in brain ECF. A BBr ($C_{br.u}/C_{bl.u}$) < 1 suggests Cl_{out} is $> Cl_{in}$. This suggests brain Al efflux is mediated by a process other than diffusion, e.g., it is mediated by carrier-mediated transport.

It was hypothesized that the monocarboxylate transporter (MCT) mediates Al citrate distribution across the BBB. Al can form coordination bonds with two carboxylates and the hydroxyl group of citrate, leav-

ing a terminal carboxylate group unbound at physiological pH [154]. Furthermore, the MCT rate of substrate transport across the BBB is more than sufficient to explain the rate of Al appearance in brain dialysate after i.v. Al citrate injection [134]. To test this hypothesis, microdialysis of both brain frontal cortices and blood was conducted in rats given an Al citrate infusion to achieve a steady state Al BBr, which averaged 0.24 [155,156]. Addition of metabolic inhibitors to the dialysate entering the brain increased the Al BBr to a value not different from one. Cyanide (an electron transport chain complex IV inhibitor in the mitochondrial respiratory chain which blocks cellular respiration) and 2,4-dinitrophenol (an uncoupler of oxidative phosphorylation that inhibits ATP synthesis without effecting mitochondrial electron transfer) were used. This suggests an energy-dependent process was mediating brain Al efflux. Addition of pyruvate (an MCT substrate) also increased the Al BBr, suggesting it competitively inhibited MCT efflux of brain Al. As the MCT is a proton co-transporter, its activity positively correlates with proton concentration. Treatments selected to reduce the brain ECF proton concentration surrounding the microdialysis probe increased the Al BBr. Although consistent with the hypothesis that the MCT mediates Al citrate efflux from the brain, there was no direct demonstration of this.

To further assess the hypothesis that the MCT is a mediator of Al citrate transport, studies were conducted with rat erythrocytes, a classical preparation to study the MCT. There was no evidence of Al ¹⁴C-citrate uptake into erythrocytes, suggesting Al citrate does not serve as an effective substrate for the MCT (isoform MCT1) or the band 3 anion exchanger [157].

To identify the characteristics, and try to identify the transporter(s) mediating Al citrate distribution across the BBB, studies were conducted using b.End5 cells, an immortalized cell line established from Balb/c mice BCECs. Intracellular Al citrate after 1 hour was ~ 25% of the medium Al citrate concentration. As diffusion would predict this to be 1%, the result suggested transporter-mediated uptake. Al citrate uptake was not sodium or pH dependent. Metabolic inhibitors (azide, 2,4-dinitrophenol and rotenone) reduced Al uptake, whereas ouabain (an inhibitor of Na/K-ATPase) did not, suggesting energy dependence that was independent of Na/K-ATPase. Lack of Al uptake inhibition by α -ketoglutarate or malonate suggested the dicarboxylic acid transporter or α -ketoglutarate exchanger were not involved. Al citrate uptake was inhibited by numerous compounds,

most of which were MCT substrates or inhibitors (benzoate, α -cyano-4-hydroxycinnamic acid [CHC], 4,4'-diiodothiocyanostilbene-2,2'-disulfonic acid [DIDS; an inhibitor of the MCT and the anion {Cl/HCO₃, band 3} exchanger], fluorescein, 3-isobutyl-1-methylxanthine [IBMX], niflumate, phloretin, probenecid, pyruvate, salicylate, and sulfobromophthalein). Based on these results, an organic anion transporter, the organic anion transporting polypeptide (oatp) family, was suggested to be a candidate. Al citrate was found to be a weak inhibitor of the uptake of *p*-aminohippurate, an organic anion transported substrate, in oocytes expressing rOAT3, suggesting it may be a substrate of an organic anion transporter (B. Feng, K.M. Giacomini & Yokel, unpublished observations). Further work will be necessary to identify with confidence the transporter(s) for Al at the BBB.

In summary, there is evidence for transporter-mediated influx and efflux of Al across the BBB (Table 5). There are proposed roles for TfR-ME and the glutamate transporter system Xc⁻ in brain uptake of Al as Al transferrin and Al citrate, respectively. There is also evidence for transporter-mediated efflux from the brain. Each of these transporter-mediated processes could potentially provide different rates of Al flux among humans, due to 1) acquired differences in transporter activity, and 2) the presence of other substances that alter the kinetics of Al due to competition with Al as a substrate or non-competitive allosteric inhibitor or facilitator of Al transport.

No reports were found addressing Al transport by the choroid plexus.

11. Manganese, Parkinson's disease and parkinsonism

Human Mn exposure can derive from several sources. The fumes released from welding rods can contain 20% or more Mn [158]. The fuel additive methylcyclopentadienyl Mn tricarbonyl (MMT), which increases octane and reduces deposit accumulation in internal combustion engines, meeting the same objective that tetraethyl Pb addressed, is primarily converted during internal combustion of gasoline to Mn phosphate and sulfate, which are released from the exhaust system into environmental air [159].

Mn can cause a parkinsonism-like syndrome, mannanism, which is initially manifest as apathy, anorexia, insomnia, extreme fatigability, somnolence, and a labile mood. It can progress to symptoms resembling

Table 5

A summary of the reported and hypothesized mechanisms of brain influx and efflux of metals suspected of contributing to neurodegenerative diseases

Metal	The mechanism(s) of brain influx	The mechanism(s) of brain efflux
Al	<ul style="list-style-type: none"> – Transport, perhaps of Al transferrin by transferrin-receptor mediated endocytosis at the BBB. – Transport, perhaps of Al citrate by system Xc⁻ at the BBB. 	<ul style="list-style-type: none"> – Transport, presumably as Al citrate at the BBB.
Cu	<ul style="list-style-type: none"> – Transport, thought to be mediated by ATP7A and perhaps ATP7B and CTR1 at the BBB. 	<ul style="list-style-type: none"> – Transport by ATP7A.
Fe	<ul style="list-style-type: none"> – Transport of Fe transferrin by transferrin-receptor mediated endocytosis at the BBB. – Transport by non-transferrin-dependent mechanisms at the BBB. – DMT1 plays a role in brain Fe uptake at the BBB. 	<ul style="list-style-type: none"> – There is no evidence for transporter-mediated efflux.
Pb	<ul style="list-style-type: none"> – By a passive process for PbOH⁺ at the BBB. – Perhaps by store-operated cation channels at the BBB. 	<ul style="list-style-type: none"> – Appears to be mediated by an ATP-dependent Ca pump (ATPase)
Mn	<ul style="list-style-type: none"> – Perhaps as the Mn ion by store-operated Ca channels, at the BBB. – Transport of Mn transferrin by transferrin-receptor mediated endocytosis at the BBB. – Transport of Mn citrate at the BBB. 	<ul style="list-style-type: none"> – Diffusion
Hg	<ul style="list-style-type: none"> – Transport as a MeHg-L- cysteine complex by the L-system. 	<ul style="list-style-type: none"> – No reported studies have addressed this.
Sn	<ul style="list-style-type: none"> – No reported studies have addressed this. 	<ul style="list-style-type: none"> – No reported studies have addressed this.
Zn	<ul style="list-style-type: none"> – Transport, perhaps by Zip1 or Zip6 at the BBB. – Transport, perhaps by a ZnT from the choroid plexus to CSF. 	<ul style="list-style-type: none"> – Transport perhaps by ZnT1 at the BBB.

parkinsonism, including speech disturbance, gait disorders, increased muscle tone, masked facies, hypokinesia, rigidity, abnormal movements and tremors and autonomic disturbances such as increased sweating and salivation. Neuropathologically, manganism differs from PD in that the basal ganglia, not the substantia nigra, are the predominant affected sites [160–162]. The clinical presentation of manganism is characterized by early involvement of speech, gait and balance, a relative absence of resting tremor, lack of asymmetry and poor response to L-DOPA; whereas PD is characterized by resting tremor, asymmetry, and response to L-DOPA. Manganism was first described in Chilean underground miners exposed to Mn dioxide by inhalation [163], and has more recently been reported in workers in a dry cell battery factory [164], children receiving long-term parenteral nutrition solutions [165], and people drinking water contaminated by buried dry cell batteries [166]. Subtle neuropsychiatric effects on reaction time, emotional state, motor function associated with alternating and/or rapid movements and hand steadiness, cognitive flexibility and olfactory perception threshold have been attributed to Mn in industrial workers producing Mn-containing alloys [158,167,168]. For Mn to produce parkinsonism, it is assumed it must enter the brain.

Owing to the similarity between manganism and PD, there has been concern that Mn can contribute to PD. An association was found in some studies of those occupationally and environmentally exposed to Mn [169–172]. On the other hand, other studies did not find a significant link between Mn exposure and PD [173–176].

12. Brain manganese influx and efflux

It has been shown that the processes mediating the brain influx of physiological Mn concentrations are primarily through the BBB and are non-saturable. Infusion of up to 11.7 μ moles (642 mcg) Mn produced plasma Mn concentrations up to 8 μ M. Mn uptake into the CP was greater than into various brain regions (Tables 2 and 3) and was saturable. However, uptake into CSF was lower than into brain (Tables 2 and 4), suggesting that Mn enters the brain primarily through the BBB [177]. After an i.v. injection of 0.46 or 0.74 mcg (0.008 or 0.013 μ moles) Mn as the chloride, a high Mn concentration was seen in the ventricles, which was attributed to accumulation in the CP [178,179]. The decline in Mn in the CP was very slow. One hour after the

injection, CSF contained 0.0053% of the dose/g compared to 0.027% in blood [178]. The authors showed increased brain Mn 6 days after the injection. Although it is not possible to assign the source to release from CP versus distribution across the BBB, the much higher Mn in the brain after 6 days compared to 1 hour suggests a slow process, which is more likely release from CP than uptake across the BBB, due to the rapidly declining blood Mn after an i.v. injection. Perfusion of carrier-free Mn or 0.008 to 4.4 μ M Mn ion, Mn citrate or Mn transferrin resulted in much greater uptake into the CP than into brain regions [180,181].

There was no evidence for CP uptake of Mn following its injection into the CSF of rats [182].

Mn is a candidate to enter the brain as a transferrin complex. However, this process is quite slow. TfR-ME processes ~ 2.5 Fe molecules/receptor/minute [183]. The presence of blood and plasma proteins, and to a greater extent transferrin, decreased brain Mn uptake [180,184]. Hypotransferrinemic mice showed similar brain Mn uptake as normal mice, although they had elevated liver Mn, suggesting the presence of non-transferrin mechanisms to distribute Mn to the brain [185]. Similarly, 1 hour and 1 week after i.v. injection of 54 Mn chloride, total brain 54 Mn was not different in hypotransferrinemic and control mice, although some regional differences in 54 Mn were seen, suggesting transferrin plays only a minor role in brain Mn uptake [186]. Brain uptake of Mn from the lateral ventricle was less after intracerebroventricular injection of Mn transferrin than the Mn ion [179]. It was concluded that non-protein bound Mn^{2+} enters the brain so much more rapidly from blood than Mn^{3+} transferrin that non-protein bound Mn^{2+} would be the predominant species entering the brain from plasma, even if it was only 1% of total plasma Mn [177]. It has been calculated that nearly 100% of plasma Mn^{+3} is transferrin bound whereas 84, 6.4, 5.8, 2 and 1.8% of Mn^{+2} is associated with albumin, water as the hydrated ion, bicarbonate, citrate and other low molecular weight ligands, respectively. These results suggest potential Mn species, other than Mn albumin (which would not cross an intact BBB to any extent), that might cross the BBB [187]. Inclusion of Fe in the perfusate containing the Mn ion did not inhibit brain Mn uptake [188].

It was suggested that DMT1 plays a role in brain Mn uptake at the BBB, just as it was believed to be involved in Fe transport into the brain. It was shown that DMT1 and the TfR are expressed and co-localized at the BBB [189], however subsequent studies did not find DMT1 expression in BCECs of one section from

monkey or Belgrade or normal rats [190,191]. DMT1 is a proton co-transporter. To test the hypothesis that it mediates brain Mn uptake, the uptake of Mn, introduced as the free ion or as a complex with transferrin, was determined in homozygous Belgrade (*b/b*) rats compared to their phenotypically normal *+/b* littermates and Wistar rats. No differences of brain Mn uptake were observed among the three groups [192]. Additionally, Mn uptake was studied in bovine BCECs at pH 6.4 to 7.9. Mn uptake increased with increasing pH, which is the opposite of that predicted if uptake was mediated by DMT1 [193]. The results of these two studies suggest uptake of the Mn ion and Mn transferrin across the BBB is not dependent on DMT1. However, studies with RBE4 cells, an immortalized cell line derived from rat BCECs, found pH dependence, which was characterized by decreasing Mn uptake with increasing pH from 7 to 7.8 [194,195].

The uptake of the Mn^{+2} ion by bovine BCECs was studied. Mn uptake was temperature dependent but not effected by the metabolic inhibitors 2,4-dinitrophenol, azide, 2-deoxyglucose, or ouabain [193]. Studies with RBE4 cells cultured in the absence and presence of astrocyte-conditioned media (ACM) also found temperature dependence and energy dependence, as cyanide and rotenone (an electron transport chain complex I inhibitor) and oligomycin ABC (an inhibitor of mitochondrial ATPase and phosphoryl group transfer) decreased Mn uptake [194,195]. Sodium replacement in the uptake medium with choline, but not lithium, significantly increased Mn uptake into bovine BCECs [193]. Similarly, sodium replacement with choline slightly, but significantly, increased Mn uptake into RBE4 cells after 60 minutes in the absence of ACM and at 10 minutes, but not later, in the presence of ACM [194,195]. Therefore, there is no evidence that Mn uptake is sodium dependent. Mn uptake by RBE4 cells was also influenced by Fe status. Both decreased Fe (produced by the chelator desferrioxamine) and increased Fe (addition of Fe dextran) increased Mn uptake [194,195]. Mn uptake by bovine BCECs was reduced by inhibitors of Ca transport, vanadate (a Ca-ATPase inhibitor) and nickel, as well as cyclopiazonic acid and thapsigargin (agents that inhibit the sarco/endoplasmic reticulum ATPase which transports Ca into the endoplasmic reticulum, a.k.a. store-operated Ca channel activators). It was concluded that both Ca-dependent (perhaps involving a store-operated Ca channel) and Ca-independent mechanisms mediate Mn^{+2} ion uptake across the BBB [193].

In summary, there appear to be multiple mechanisms mediating Mn brain uptake across the BBB, including

uptake of the Mn transferrin complex presumably by TfR-ME, as well as more than one process to take up Mn that is not associated with transferrin. However, a significant role of DMT1 has not been shown.

The efflux of Mn from the brain was assessed in one study. The brain efflux index method [196] was used to determine the rate of Mn ion and Mn citrate brain efflux. This method is based on the rate of reduction in the brain of the test substance (Mn) and a substance that very slowly diffuses across membranes, including the BBB. Both sucrose and dextran were used as these relative impermeants. The rate of decrease of the Mn concentration in the brain was slower than sucrose and dextran. The predicted rate of Mn diffusion across membranes was slower than sucrose or dextran, based on their molecular weight and hydrophilicity. The interpretation of the results was that the rate of Mn efflux from the brain was consistent with diffusion, e.g., there was no evidence for transporter-mediated brain Mn efflux [197]. This is consistent with an observation that brain uptake of Mn was unidirectional [184].

In summary, there is very good evidence that transporters mediate the influx of the Mn²⁺ ion, Mn citrate and Mn Tf into the brain. Results suggest that diffusion mediates brain Mn efflux. In light of transporter-mediated brain influx but not efflux, it would be anticipated that repeated, excessive, Mn exposure might result in brain Mn accumulation over time. This has been observed in animals [198]. Similarly, the human brain Mn concentration rose in humans from infancy to adulthood and the highest Mn concentrations were in the basal ganglia, the site of Mn-induced neurotoxicity [199].

13. Brain iron influx and efflux

Iron appears to enter most cells, including those that comprise the BBB, by TfR-ME [200–202]. The brain entry rate of ⁵⁹Fe was not significantly different from transferrin [203]. However, ⁵⁹Fe influx into the brain was greater than into CSF [148]. There are estimates of 35,000 and 100,000 high affinity transferrin receptors per bovine BCEC, based on the binding of ¹²⁵I-holotransferrin [204,205]. The K_{in} values in Table 2 correspond to ~ 0.02 pmoles/sec/gm. Transcytosis of ⁵⁵Fe transferrin was reduced by non-radiolabeled Fe transferrin and by transferrin receptor antibodies (OX-26 and IgM R17 208), supporting the role of TfR-ME in brain Fe uptake [205–207]. After bolus i.v. injection of ⁵⁹Fe transferrin in the rat or perfusion of the

rat brain with transferrin-horse radish peroxidase conjugate, brain ⁵⁹Fe uptake was much greater than transferrin. Transcytosis of the conjugate was not seen, suggesting deposition of Fe in brain endothelial cells and recycling of apotransferrin to the blood [208–211]. In contrast, after accumulation of ¹²⁵I-holotransferrin in bovine BCECs and washing to remove unbound tracer, further incubation resulted in release of 10% of the ¹²⁵I-holotransferrin to the luminal and 75% to the abluminal side of the cells [205], leading the authors to conclude that most of the endocytosed holotransferrin distributed across the endothelial cells. Further studies with ⁵⁹Fe-¹²⁵I-labeled-transferrin supported this [205]. The authors found very little evidence for degradation of holotransferrin, concluding that it was transported across bovine BCECs without degradation.

Iron was considerably lower and brain transferrin somewhat lower in the brain and other organs of Belgrade rats (*b/b*), which have a DMT1 mutation, suggesting this mutation altered the structure and/or function of Fe membrane transporter(s) [191,212]. Because this genetic mutation results in reduced gastrointestinal Fe absorption, the reduced organ Fe could reflect lower circulating Fe. Iron was visualized in the BCECs, neurons, astrocytes and oligodendrocytes of Belgrade rats compared to heterozygous (*+/b*) littermates and wild-type (*+/+*) rats. Although there was less Fe in the brain cells of rats, there was no difference in the BCECs [213], suggesting DMT1 plays a role in Fe transport across the BBB. The lack of DMT1 in BCECs in *b/b*, *+/b* and *+/+* rats suggested the reduced brain Fe uptake is due to reduced neuronal Fe uptake, rather than a DMT1 defect at the BBB [191].

In summary, at the BBB, diferric transferrin (holotransferrin) binds to the transferrin receptor which is internalized into an endosome. The pH is decreased in the endosome to release the Fe from transferrin. The latter is recycled to blood. It has been suggested that DMT1 mediates the transport of Fe(II) out of the endosome [214,215]. However, the lack of demonstration of DMT1 in BCECs is not consistent with this hypothesis.

Melanotransferrin (p97) is another member of the family of Fe-binding proteins that includes transferrin. It has ~ 38% sequence identity with transferrin and has been shown to bind Fe. It was shown to increase brain Fe uptake 8- to 9-fold more than transferrin, which was more active than citrate, after i.v. injection in the mouse. It was suggested that it might mediate non-transferrin brain Fe uptake [216]. However, in the rat it delivered only 10% as much Fe to the brain as did transferrin [217]. Based on their results and the

much lower concentration of melanotransferrin than transferrin in plasma, the authors suggested it does not play a significant role in brain Fe uptake.

It was suggested that low molecular weight metal complexes may contribute significantly to brain Fe uptake [148]. There is evidence for delivery to the brain of non-Tf-bound Fe. In normal mice, brain $^{59}\text{Fe}^{+3}$ uptake after 2 hours of i.v. infusion of $^{59}\text{Fe}^{+3}$ ascorbate or ^{59}Fe transferrin was not different [207], although the 1 to 2 nmoles of injected non-transferrin bound ^{59}Fe administered may have bound to transferrin in the plasma to enable its brain uptake as ^{59}Fe transferrin. Brain uptake of $^{59}\text{Fe}^{+3}$, introduced as a two-hour infusion of $^{59}\text{Fe}^{+3}$ ascorbate, was 80- to 100-fold greater in hypotransferrinemic mice, which had < 2.5% as much serum transferrin as controls, suggesting the presence of transferrin-independent mechanism(s) of brain Fe uptake [207]. The authors suggested the Fe species taken up by the brain may be the citrate. However, brain uptake of $^{59}\text{Fe}^{+3}$ was not seen after 1 minute perfusion with $^{59}\text{Fe}^{+3}$ or $^{59}\text{Fe}^{+3}$ citrate. When the perfusate was buffered to pH 7.4 and the Fe was reduced to $^{59}\text{Fe}^{+2}$, brain uptake was 2-fold greater than $^{59}\text{Fe}^{+3}$. No uptake of $^{59}\text{Fe}^{+3}$ citrate was observed. It was concluded that $^{59}\text{Fe}^{+2}$ was taken up by the brain in the absence of transferrin [218]. On the other hand, brain Fe uptake was not different in hypotransferrinemic compared to normal mice 2, 6 and 24 hours after i.v. injection of ^{59}Fe chloride or 24 hours or 7 days after s.c. $^{59}\text{Fe}^{+3}$ chloride injection, consistent with the suggestion of the presence of non-transferrin mechanisms to distribute Fe to the brain [185,219]. Total brain ^{59}Fe in hypotransferrinemic mice was not different from controls 1 hour and 1 week after i.v. injection of ^{59}Fe chloride. However, nearly all of the Fe in the hypotransferrinemic mice was in the CP whereas the control brain showed considerable Fe, suggesting a role for transferrin in brain Fe uptake and distribution, and perhaps distribution from CP into CSF [186]. Twenty-four hours after s.c. injection of ^{59}Fe to 7-day old hypotransferrinemic mice, that had not received transferrin injections, and normal mice, lateral ventricle ^{59}Fe was ~ 7-fold greater in the hypotransferrinemic mice, but brain levels were lower [220]. Using bovine retinal endothelial cells as a model of BBB cells, it was found that Fe is normally transported by transferrin- and non-transferrin-dependent mechanisms [215]. In the presence of excess Fe, there was decreased transport of non-transferrin-bound-Fe, suggesting regulation of this process. In view of the lack of measurable transferrin in the plasma of hypotransferrinemic

mice, it appears that a non-transferrin mechanism can mediate brain Fe uptake. It was pointed out that in the absence of information on the cellular or subcellular localization of Fe in the brain, it is not known whether non-transferrin mechanisms deliver Fe to the brain as effectively as transferrin-dependent mechanisms [219]. Using the *in situ* brain perfusion technique, comparable K_{in} values for brain Fe influx were obtained in the absence and presence of transferrin [221], although the rates reported in this study are considerably higher than previous studies (Table 2). Iron uptake (K_{in}) into cerebral capillaries in the absence and presence of transferrin (303 and 227×10^{-5} ml/sec/gm) was much greater than into capillary-depleted brain (3 and 2.5×10^{-5} ml/sec/gm), respectively, suggesting sequestration of Fe by BCECs followed by slow release into the brain [221]. Similarly, the K_{in} of iron into the CP was not different in the absence or presence of transferrin (Table 3), and uptake into CSF was very much less than into the CP (0.28 vs. 17 in the absence and 0.15 vs. 22×10^{-5} ml/sec/gm in the presence of transferrin), suggesting sequestration of Fe in the CP [221].

There are no published studies that assessed the mechanism or rate of Fe efflux from the brain [219]. Clearance of Fe and transferrin from the brain following lateral ventricular injection has been reported [203]. Approximately 75% of the injected transferrin but only 5 to 10% of the Fe was in blood 1 hour after injection, suggesting clearance of the former into blood and the latter via CSF turnover. It was concluded that the bulk flow of CSF out of the brain was sufficient to account for the efflux of Fe from the brain [214]. This was based on comparison of brain Fe influx, calculated from the influx rate constant times serum Fe concentration, to CSF outflow from the brain times CSF Fe concentration. Iron clearance in CSF was approximately the same as its influx across the BBB.

14. Brain copper influx and efflux

As noted above, inheritance of a gene mutation for ATP7B has been shown to contribute to Wilson's disease. Menkes disease is associated with inheritance of a mutation of a similar Cu transporter (ATP7A). It has been shown that murine BCECs express ATP7A, which mediates Cu efflux [24], but protein expression was not seen beyond post-natal day 4 [36].

Histidine, and to a lesser extent other amino acids, increased Cu uptake into hypothalamic slices, by a pro-

cess that appeared to be carrier-mediated facilitated diffusion [222,223].

Copper efflux from murine BCECs was blocked by an agent that is a potential inhibitor of ATP7A [24]. These results have been interpreted to suggest that Menkes disease is due to lack of Cu transport into the brain at the BBB by the mutated ATP7A in the BCECs [24].

The plasma membrane transporter Ctr1, which takes up Cu(I), is expressed in murine brain capillaries and rat choroid plexus [224,225].

15. Brain zinc influx and efflux

The clearance of ^{65}Zn , as the chloride, from blood to brain across the BBB was determined using the i.v. infusion and i.v. bolus injection methods (Table 2) [226–229]. Addition of L- and D-histidine increased Zn uptake at the BBB \sim 12-fold (Table 2) and perhaps at the CP-CSF barrier [229–231]. Brain Zn concentrations were greater after intracerebroventricular injection of Zn histidine than Zn chloride [231]. Computation of the results of Zn uptake from varied concentrations of Zn and histidine and the formation constants of Zn histidine species suggested that the Zn species was Zn histidine $^+$. Addition of L-phenylalanine, a substrate for the L transporter, and L-arginine, a substrate for the γ^+ transporter, did not affect Zn uptake [229]. The authors speculated that the low molecular weight complex with histidine facilitates the diffusion of Zn through unstirred water layers to enhance its transport, although the mechanism of trans-membrane flux was not suggested. Additionally, Zn increased rat BCEC L- and D-histidine uptake [232,233]. The metabolic inhibitors 2,4-DNP and rotenone did not reduce zinc enhancement of L-histidine uptake [223]. Similarly, Zn flux across porcine BCECs was not affected by the energy inhibitors azide, fluoride or 2,4-DNP, nor was it affected by the presence of Al, cadmium, Cu, Mn or Pb [234]. Zinc uptake was similar in the presence of a range of Zn concentrations in the uptake medium, suggesting saturation of Zn uptake. When these cells were grown in a medium deficient in Zn, uptake and flux of Zn across the cells increased. When grown in a medium with a moderate Zn excess, Zn uptake into BCECs increased but transport across the cells decreased [235]. These results suggest a transport process that is highly selective for Zn.

Two families of Zn transporters have been identified that are believed to be involved in Zn distribu-

tion across the plasma membrane. Members of the Slc30a/SLC30A family (ZnT and ZNT), previously called the cation diffusion facilitator family, are thought to selectively mediate Zn transport from the cytoplasm out of the cell or into intracellular compartments [236, 237]. ZnT1, which is expressed at the plasma membrane, is believed to efflux Zn from cells. Members of the Slc39a/SLC39A family (Zip and ZIP), facilitate Zn uptake into cells and perhaps transport of Zn from vesicles to cytoplasm [236,237]. Zip1 mediates cellular Zn uptake [237]. No reports were found demonstrating the expression of ZnT1, ZnT3, ZnT4 or ZnT6 in the cells comprising the BBB. Zip6 expression was seen in BCEC cells [238].

No reports were found addressing zinc efflux from the brain.

After an i.v. injection of 8 mcg Zn, as the chloride, a high Zn concentration was seen in the ventricles, which was attributed to accumulation in the CP [178]. One hour after the injection there was 0.018% of the dose/gm CSF and 0.25%/gm blood. Brain Zn was higher 1 day after the injection. However, it is unknown if the source of brain Zn was release from the CP or distribution across the BBB. ZnT1, ZnT2, ZnT3 and ZnT6 were expressed by CP epithelial cells [239]. ZIP6 expression was seen on the apical membrane of CP epithelial cells [238]. As the ZnTs are thought to mediate cellular efflux from the cytoplasm, one or more may transport Zn from the CP to CSF.

16. Brain lead influx and efflux

An observation suggested that colloidal Fe hydroxide adsorbed Pb to reduce its brain uptake. This hypothesis was supported by the observation that concurrent i.v. infusion of Fe^{3+} or Al chloride with ^{203}Pb greatly reduced brain Pb uptake, suggesting Pb that enters the brain is the free ion or is a complex with small molecular weight ligands [240]. Addition of albumin, L-cysteine or EDTA essentially abolished brain Pb uptake. Albumin does not appreciably cross an intact BBB. In plasma, \sim 40% of Pb is bound to albumin and the remainder to cysteine and other thiol-containing ligands, leaving a free Pb concentration of $\sim 10^{-12}$ M [241]. In contrast, addition of Ca or a Ca channel blocker to the perfusate, varying bicarbonate concentration, replacement of sodium with choline, replacement of potassium with sodium, and addition of DIDS and barium in the perfusate did not affect brain Pb uptake. Vanadate, carbonyl

cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, which uncouples mitochondrial oxidative phosphorylation by collapsing the proton electrochemical gradient, a protonophore that can test for pH dependence), and stannic chloride (stannic ions may compete with Pb as a transporter substrate) significantly increased brain Pb uptake. In contrast, *p*-chloromercuribenzenesulfonate (PCMBs, which modifies cysteine residues), significantly reduced brain Pb uptake. Greater brain Pb uptake was seen as pH increased from 6.6 to 7.8 whereas increasing potassium decreased Pb uptake. Based on these results, and the predicted chemical speciation of Pb as a function of pH, the authors suggested that Pb may cross the BBB as an ion, PbOH^+ , by a passive (non-energy-dependent) process [240]. The results with potassium and barium suggested that Pb uptake was dependent on net positive charge movement. It has been observed that depletion of intracellular Ca stores increased Pb uptake into bovine BCECs [242]. The depletion was produced by thapsigargin, cyclopiazonic acid and *tert*-butylhydroquinone (sarco/endoplasmic reticulum ATPase inhibitors). This causes depletion of Ca stores, stimulating uptake of extracellular Ca through voltage-insensitive Ca channels. These results suggest Pb uptake may be mediated by store operated cation channels.

Using the *in situ* brain perfusion method, K_{in} values were obtained that were considerably greater than obtained in studies that used Pb infusion for up to 4 hours to produce a relatively constant plasma Pb concentration (Table 2) [240,241,243]. The authors noted that uptake decreased after 1 minute of *in situ* brain perfusion, which may be due to Pb efflux from the brain [243]. The increased efflux of brain Pb with time and the complexation of Pb by components of blood during prolonged Pb infusion may explain the much smaller K_{in} values obtained with the prolonged infusion method.

An ATP-dependent Ca pump (ATPase) at the BBB was suggested to efflux Pb from the brain, based on the results with vanadate, FCCP and stannic ion [240].

After i.v. injection of ^{210}Pb nitrate, Pb concentrations in the CP were ~ 70 -fold greater than in brain, suggesting the CP may act as a sink to reduce Pb distribution into CSF and brain. The CSF Pb concentration was $\sim 10\%$ of brain Pb [244], suggesting the CP effectively complexed Pb and that the BBB is the major site of brain Pb entry. A more recent study found brain to have ~ 2 -fold more Pb than CSF (Tables 2 and 4). The higher CP than brain Pb concentration was replicated by [62] who found ~ 60 -fold higher Pb in the CP than the brain after its i.p. injection.

17. Brain mercury influx and efflux

Brain Hg is higher after exposure to elemental than inorganic Hg [245]. It is thought that this is due to its high lipophilicity, enabling diffusion across the BBB. The active transport of MeHg, as a cysteine complex, was reported in the gut [246]. The concurrent administration of L-cysteine with MeHg increased brain Hg uptake 3-fold [247,248]. Using the Oldendorf single pass model, enhanced brain Hg uptake was found to be inhibited by L-cysteine-L-methionine, but not D-cysteine [248]. A similar enhancement of MeHg uptake by L-cysteine was seen in isolated bovine BCECs [249]. L-methionine, a substrate for neutral amino acid transport, inhibited the enhanced uptake, supporting the suggestion that uptake was transporter-mediated, which the authors suggested might be the L-system [249]. Further proofs of this hypothesis were 1) the demonstrations that MeHg-L-cysteine brain uptake was saturable, inhibited not only by L-methionine but by an L system amino acid analog 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid, but not by a substrate for the other large neutral amino acid transporter at the BBB, the alanine-preferring system, α -aminoisobutyric acid, and 2) that Me-Hg-L-cysteine, but not Me-Hg chloride, inhibited L-methionine brain uptake [250]. Me-Hg-glutathione, which is metabolized to Me-Hg-L-cysteine, also resulted in significant brain Hg uptake, suggesting that it was the plasma source of Me-Hg-L-cysteine [250].

Although the efflux of Hg or MeHg from the brain has apparently not been studied, it appears that the same transporter for MeHg-L-cysteine at the BBB mediates its influx and efflux in astrocytes [251,252]. This raises the possibility that this transporter has the same ability at the BBB, where it is bidirectionally active [253]. However, MeHg is metabolized to inorganic Hg in the brain where it is "trapped", perhaps because of its hydrophilicity and inability to serve as a substrate for an efflux transporter at the BBB.

The CP seems to protect the brain from Hg exposure based on the 5- to 12-fold higher Hg concentration there than in brain cortex after i.p. injection of methyl and inorganic Hg, respectively, and even higher CP:CSF ratios [62].

18. Brain organotin influx and efflux

Organotins are model compounds to produce neurotoxicity and have produced human poisoning. They have not been significantly implicated in common neu-

rodegenerative diseases. Triethyltin penetrates the BBB to produce brain edema due to fluid accumulation in vacuoles at the inter-period line of CNS myelin, resulting in demyelination [254,255]. Trimethyl tin causes neuronal degeneration, mediated in large part by oxidative damage [256,257]. The organotins are quite lipophilic. Studies have shown that BBB permeability is not increased at the time of brain entry of organotins [255,258,259]. These results suggest that the organotins are not impairing BBB integrity to facilitate their distribution through the paracellular pathway between the endothelial cells that comprise the BBB. The organotins might be crossing the BBB by the trans cellular route, e.g., through the endothelial cells due to their lipophilicity, or aided by a transporter. No reports were found that describe the rate of BBB permeation of the organotins or that address the mechanism(s) of brain entry.

19. Summary of metal flux across the blood-brain barrier

Transporters that mediate BBB transport of a few metals have been identified. TfR – ME mediates Fe distribution across the BBB, and perhaps Al and Mn. ATP7A and ATP7B, which play a role in Cu imbalance diseases, and at least one of which is expressed at the BBB, probably transport Cu there. Methyl Hg, when complexed to cysteine, can serve as a substrate for the L system. For some metals, there is good evidence for transporter-mediated flux across the BBB, but the transporter(s) are not yet well characterized. Although there are suggestions that TfR-ME, Oatp2 and the glutamate transporter Xc⁻ contribute to the flux of Al across the BBB, there is no definitive demonstration for any of these. Similarly, the suggestions that TfR-ME, store operated cation channels and probably other processes mediate brain Mn uptake are lacking definitive proof. Mechanisms to transport Ca, such as store operated cation channels, have also been suggested to mediate distribution of Pb across the BBB, but definitive demonstration is lacking. Although BBB, and perhaps CP, flux of Zn seems to involve a transporter for which histidine is a substrate, its identity is unknown and has evidently not been suggested. Finally, very little is known about how some other neurotoxic metals, such as the organotins, cross the BBB.

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