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Astrocytes in Heavy Metal Neurotoxicity and Neurodegeneration

Baoman Li¹, Maosheng Xia², Robert Zorec³, Vladimir Parpura⁴ and Alexei Verkhatsky^{1,5,6*}

¹Practical Teaching Centre, School of Forensic Medicine, China Medical University, Shenyang, People's Republic of China.

²Department of Orthopaedics, The First Hospital, China Medical University, Shenyang, People's Republic of China.

³Celica BIOMEDICAL, Tehnološki Park 24, 1000 Ljubljana, Slovenia; Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia.

⁴Department of Neurobiology, The University of Alabama at Birmingham, Birmingham, AL 35294, USA.

⁵Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK.

⁶Achucarro Center for Neuroscience, IKERBASQUE, Basque Foundation for Science, 48011 Bilbao.

Corresponding authors:

Prof. Baoman Li, Ph.D., M.D.

Practical Teaching Center, School of Forensic Medicine, China Medical University
No. 77, Puhe Street, Shenbei District, Shenyang, P.R. China

Postcode: 110177

Phone: 86(24)31939437

Fax: 86(24) 83282772

Email: bmli@cmu.edu.cn

OR

Prof. Alexei Verkhatsky, Ph.D., M.D.

Faculty of Biology, Medicine and Health

The University of Manchester

Manchester M13 9PT, UK

Email: Alexej.Verkhatsky@manchester.ac.uk

Abstract

With the industrial development and progressive increase in environmental pollution, the mankind overexposure to heavy metals emerges as a pressing public health issue. Excessive intake of heavy metals, such as arsenic (As), manganese (Mn), mercury (Hg), aluminium (Al), lead (Pb), nickel (Ni), bismuth (Bi), cadmium (Cd), copper (Cu), zinc (Zn), and iron (Fe), is neurotoxic and it promotes neurodegeneration. Astrocytes are primary homeostatic cells in the central nervous system. They protect neurons against all types of insults, in particular by accumulating heavy metals. However, this makes astrocytes the main target for heavy metals neurotoxicity. Intake of heavy metals affects astroglial homeostatic and neuroprotective cascades including glutamate/GABA-glutamine shuttle, antioxidative machinery and energy metabolism. Deficits in these astroglial pathways facilitate or even instigate neurodegeneration. In this review, we provide a concise outlook on heavy metal-induced astrogliopathies and their association with major neurodegenerative disorders. In particular, we focus on astroglial mechanisms of iron-induced neurotoxicity. Iron deposits in the brain are detected in main neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. Accumulation of iron in the brain is associated with motor and cognitive impairments and iron-induced histopathological manifestations may be considered as the potential diagnostic biomarker of neurodegenerative diseases. Effective management of heavy metal neurotoxicity can be regarded as a potential strategy to prevent or retard neurodegenerative pathologies.

Keywords: heavy metals; astrocytes; neurotoxicity; neurodegeneration; glutamate

Highlights

Astrocytes protect neurones against neurotoxicity induced by heavy metals.

Astrocytes are the main target for heavy metal neurotoxicity.

Heavy metals affect astrocytic plasmalemmal glutamate transporters resulting in neurodegeneration.

Iron overload triggers neurodegeneration by inducing astrogliopathies.

Over-intake of heavy metal(s) including iatrogenic sources should be carefully managed.

1. Introduction

Humans are exposed to pathological load of metals through contaminated food, contaminated environment (water and air) or through occupational exposure (Liu et al., 2019; Bai et al., 2019; Jan et al., 2015; Qu et al., 2012; Sarah et al., 2019); excess of metals in the body often results in neurotoxicity and associated neurological disorders. Excessive accumulation of metallic elements, such as arsenic (As), manganese (Mn), mercury (Hg), lead (Pb), aluminium (Al), nickel (Ni), bismuth (Bi), cadmium (Cd), zinc (Zn), copper (Cu) and iron (Fe), are known to be neurotoxic and increase the risk for neurodegenerative diseases, particularly of Alzheimer's disease (AD) and Parkinson's disease (PD) (Supino-Viterbo et al., 1977; Kirkley et al., 2017; Pamphlett and Kum, 2018; Rahman et al., 2019; Ijomone et al., 2020; Sudhakaran et al., 2019).

Metal poisoning has been recognized throughout history of mankind. Lead toxicity, which results in memory impairments, has been known since antiquity (Waldron, 1973), while adverse effects caused by water drinking from lead-furnished water pipes have been proposed as a biological background for the fall of the Roman empire (Nriagu, 1983). The first clinical description of an acute Pb poisoning that included paralysis was produced by Nicander of Colophon in the 2nd century BC (Waldron, 1973). Chronic mercury poisoning caused erethism mercurialis (also known as mad hatter syndrome in the 17th century France) characterised with psychotic symptoms and impaired memory (O'Carroll et al., 1995). Mercury poisoning resulting in neurodegeneration was observed on the shores of Minamata bay, which gave the name to Minamata disease (McAlpine and Araki, 1958). Today, metal pollution of food chains rise significant concerns (Rahman et al. 2014; Wang et al. 2017; Wu et al., 2013). In particular iron oxides are ubiquitous in both natural and industrial environments, with iron being fundamental for metabolic and physiological activities. At the same time, a loss of iron homeostasis or an excessive load with iron (resulting from dietary intake or from medical procedures such as iron implants or usage of iron oxide nanoparticles) is harmful for health (Okerefor et al., 2020; Li Y. et al., 2020; Vogel et al., 2016; Xia et al., 2020).

Metal toxicity affects the brain development and impairs cognition, memory and learning in humans and in animal models (Hussien et al., 2018; Mason et al., 2014). Clinically, chronic superfluous intake of metals usually instigate neurological symptoms such as dizziness, headaches, motor impairments and cognitive and memory deficits (Wu et al., 2013; Peres et al., 2015). These symptoms may also signal the beginning of a neurodegenerative process (Bauer et al., 2015; Buckner, 2004), which results from integration of multiple factors, including genetic background, adverse lifestyle and environmental pressures (Eid et al., 2019). Levels of some heavy metals such as Mn, Hg and Cd are increased in the plasma and cerebrospinal fluid (CSF) of AD-patients (Gerhardsson et al., 2008; Basun et al., 1991). Heavy metals have been reported to increase the presence of AD-relevant

proteins, such as β -amyloid, Tau and ApoE4 both *in vitro* and *in vivo* (Moyano et al., 2020; Godfrey et al., 2003; Olivieri et al., 2000; Al Kahtani et al., 2020). In addition, heavy metals elevate β -amyloid load by decreasing the clearance of β -amyloid from the brain (Gu et al., 2011; Kim et al., 2014). Recent and growing evidence reports that disturbed iron homeostasis is an early presentation in the AD (Kim et al., 2018; Mandel et al., 2007), while the level of iron in the hippocampus and cortex of AD-patients is increased compared with healthy subjects (Corrigan et al., 1993; Gu et al., 1998). Abnormal level of iron causes the suppression of function of several enzymes that require iron as a co-modulator, forming toxic oxidizing species, and stimulating β -amyloidogenesis (Qian and Wang, 1998). Several common genetic polymorphisms that cause the aberrant iron homeostasis are frequently associated with AD (Crespo et al., 2014; Nandar and Connor, 2011), although the mechanistic links remain unclear. Environmental overexposure to Pb is another well known risk factor for AD (Chin-Chan et al., 2015). In animal models, the oral intake of excessive Pb increases the cerebral level of β -amyloid, as well as levels of the pro-inflammatory interleukin-1 (IL-1) and tumour necrosis factor α (TNF- α); these changes were associated with the impaired cognitive capacity (Li et al., 2014a).

The over-intake of heavy metals represents a high risk for PD, resulting from the loss of dopaminergic neurones in the substantia nigra (Mu et al., 2020). Significant association between PD and exposure to Cu, Mn and Fe in workers with more than 20 years of occupational history has been identified in a population-based case-control study in Detroit (Gorell et al., 1997; Powers et al., 2003; Fukushima et al., 2013). In animal models, chronic exposure to Mn, Cd or Hg triggers neuroinflammation and impairs the function of mitochondria, thus, producing the PD-like neurological symptoms (Hammond et al., 2020; Zhang et al., 2017; Qu et al., 2013; Han et al., 2017). Substantia nigra pars compacta has the highest levels of iron in the human brain and, hence, iron is considered as a risk factor for PD (Jiang et al., 2019). In PD patients, iron levels are increased in parietal and prefrontal cortices; increase in iron can be a predictor of poor cognitive outcome, and its elevation in the putamen predicts poorer motor function (Thomas et al., 2020). Overload with iron triggers production of reactive oxygen species (ROS) and pro-inflammatory factors, thus, further exacerbating neuroinflammation and brain pathology (Bjørklund et al., 2019; Heneka et al., 2010; Neal and Richardson, 2017).

Astrocytes are the homeostatic cells of the central nervous system (CNS); in particular they are fundamental for the ionostasis of the nervous tissue (Verkhratsky and Nedergaard, 2018). Through an extended family of plasmalemmal transporters astrocytes control concentrations of ions in the interstitial fluids (Verkhratsky and Rose, 2020; Rose and Verkhratsky, 2016). In particular, astroglial transporters remove excess of heavy metals from the brain parenchyma, thus, protecting neurones against toxicity. Of note, microglial cells also contribute to this protection (Zheng et al., 2010). Accumulation of heavy metals, however, damages astrocytes and affects their homeostatic and neuroprotective cascades, of which most important are associated

with glutamate-glutamine transport and anti-oxidative support.

2. Astrocytes glutamate homeostatic cascade as the main target for metal neurotoxicity

2.1. The homeostasis of glutamate

Glutamate and γ -aminobutyric acid (GABA) are respectively major excitatory and inhibitory neurotransmitters in the brain. Both share the same biosynthetic pathway deriving from glucose, which makes catabolism of them strictly astroglia-dependent. Astrocytes are the only cells in the brain capable of producing *de novo* glutamate (and by proxy, GABA) from glucose (Hertz et al., 1999). The key enzymes for this process are pyruvate carboxylase (which produce α -ketoglutarate) and glutamine synthetase (which converts glutamate to glutamine); both these enzymes are expressed exclusively in astrocytes (Norenberg and Martinez-Hernandez, 1979; Schousboe et al., 2014; Shank et al., 1985; Rose et al., 2013). Glutamine is a non-toxic precursor for glutamate and hence it can be safely transported to neurones where it is converted (by phosphate-activated glutaminase) to glutamate in excitatory terminals (Hertz, 2013); in inhibitory terminals glutamate is converted to GABA (Rose et al., 2013). This final conversion is mediated by glutamate decarboxylase (Bak et al., 2006). These enzymatic cascades are coordinated with astroglial plasmalemmal transporters and operate in concert as an astroglial glutamate/GABA-glutamine shuttle, which controls extracellular levels of glutamate and supplies neurones with glutamine. Control over extracellular glutamate is of paramount importance to prevent glutamate excitotoxicity which appears as the major neuronal killer in conditions of brain pathology (Choi, 1992).

Glutamate, secreted during neurotransmission is taken up by astrocytes via sodium-dependent excitatory amino acid transporters 1 and 2 (EAAT1/SLC1A3 and EAAT2/SLC1A2, also known, in rodent experiments as glutamate-aspartate transporter GLAST and glutamate transporter-1, GLT1). These transporters are almost exclusively astroglial with some variations between brain regions (Danbolt, 2001; Zhou and Danbolt, 2013); activity of these transporters is regulated by transmembrane Na^+ gradients (Kirischuk et al., 2007). Expression of glutamate transporters varies across the brain; the EAAT1 dominates cerebellum, retina and circumventricular organs (Lehre and Danbolt, 1998; Berger and Hediger, 2000; Rauen et al., 1996), whereas EAAT2 demonstrates higher expression in other regions. The average density of astroglial transporters is exceptionally high with EAAT1 reaching $4700/\mu\text{m}^2$ in Bergmann glia and $2300/\mu\text{m}^2$ in astrocytes of the CA1 hippocampal area; the density of EAAT2 is $\sim 8500/\mu\text{m}^2$ in the hippocampus and $\sim 740/\mu\text{m}^2$ in the cerebellum (Lehre and Danbolt, 1998). At the ultrastructural level most of transporters are concentrated at the perisynaptic astroglial processes (Chaudhry et al., 1995). After entering astrocytes, glutamate is mainly converted to glutamine by glutamine synthetase; glutamine is subsequently transported to neurones. This transport is mediated by

sodium-coupled neutral amino acid transporters; astroglial SNAT3/SLC38A3 and SNAT5/SLC38A5 mediate export of glutamine, while neuronal SNAT1/SLC38A1, SNAT2/SLC38A2 and SNAT4/SLC38A4 are responsible for glutamine import (Verkhratsky and Nedergaard, 2018; Verkhratsky and Rose, 2020). Proper functional activity of glutamate/GABA-glutamine shuttle is critical for neurotransmission and changes in expression or activity of its components lead to various pathologies including neurodegeneration (Rose et al., 2013).

2.2. Astrocytes define glutamate excitotoxicity

In pathological conditions, decreased expression and/or inefficiency of EAATs results in the increased level of extracellular glutamate with the subsequent excitotoxicity (Olloquequi et al., 2018). Mechanistically, an excess of extracellular glutamate depolarises neurones, which leads to opening of Ca^{2+} permeable NMDA receptors and voltage-gated Ca^{2+} channels, subsequently causing an overload of the cytoplasm with Ca^{2+} . This pathological Ca^{2+} signalling initiates oxidative stress, mitochondrial damage, massive activation of proteolytic enzymes etc., ultimately instigating necrotic or apoptotic cell death (Peng and Jou, 2010; Martinez-Ruiz et al., 2011; Dzialo et al., 2013; Piña-Crespo et al., 2014). Excitotoxicity contributes to neurodegenerative diseases. For example, neurotoxic β -amyloid may increase extracellular glutamate, thus, triggering pathological Ca^{2+} signalling (Busche et al., 2008; Kuchibhotla et al., 2008). Pharmacological inhibition of NMDA receptors with memantine was reported to normalise cognitive performance, decrease β -amyloid load and plaque deposition in clinical and animal studies of AD (Danysz and Parsons, 2012). In PD, the expression and function of EAAT1 is significantly decreased, thus, instigating excitotoxicity and subsequent death of dopaminergic neurones (Sominsky et al., 2015). Aberrant Ca^{2+} signalling and failed $[\text{Ca}^{2+}]_i$ homeostasis induced by excitotoxicity can lead to the malfunction of mitochondrial bioenergetics and the increased level of ROS, damaging dopaminergic neurones (Cieri et al., 2017; Surmeier et al., 2017). Excitotoxicity is the key pathogenetic step in amyotrophic lateral sclerosis (ALS); in this pathology excessive glutamate in the extracellular space results from substantial down-regulation of astroglial EAAT2 transporters that results in the insufficient glutamate uptake (Mathis et al., 2017). Treatment with riluzole (2-amino-6-trifluoromethoxy benzothiazole; the only partially effective monotherapy in ALS) counteracts glutamate excitotoxicity by inhibiting glutamate release from presynaptic terminals and up-regulating expression of astroglial plasmalemmal glutamate transporters (Zarei et al., 2015; Carbone et al., 2012).

2.3. Heavy metals affect astroglial plasmalemmal glutamate transporters, hence, instigating neurodegeneration

In the brain, Mn ions ($\text{Mn}^{2+}/\text{Mn}^{3+}$) cross the blood-brain barrier (BBB) and are preferentially accumulated by astrocytes through the plasmalemmal divalent metal transporter-1 (DMT1) and by binding to and internalising with transferrin receptor

(TFR) (Fitsanakis et al., 2006 and 2007; Erikson and Aschner, 2006). The main pathological effect of Mn is the disturbance of the glutamate/GABA-glutamine shuttle at multiple levels. Excess of intra-astroglial Mn decreases the activity of glutamine synthetase and down-regulates expression of EAAT1 and EAAT2 (Deng et al., 2012; Lee et al., 2013; Johnson et al., 2018). Suppression of plasmalemmal glutamate transporters expression in astrocytes is mediated by a transcription factor Yin Yang 1 (YY1) activated by the Mn-sensitive nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) cascade (Karki et al., 2014; Karki et al., 2015). As a result, dysfunctional glutamine catabolism and elevated extracellular glutamate induce excitotoxicity, neuronal damage and contribute to neurodegenerative process (Sidoryk-Wegrzynowicz et al., 2013; Miyata et al., 1983; Lu et al., 2018). Astrocytic plasmalemmal glutamate transporters are similarly vulnerable to other heavy metals, such as Pb and Hg, both of which dysregulate glutamate homeostasis by inhibiting the expression and/or function of astroglial plasmalemmal glutamate transporters (Struzynska et al., 2005). Hippocampal structures seem to be more sensitive to Pb, which evokes deficits in cognition and learning (Gilbert et al., 1999). Additionally, the overexposure to Hg inhibits the mRNA expression and the related functions of EAATs by stimulating astrocytic ROS (Allen et al., 2001; Mutkus et al., 2005). Similarly, arsenite inhibits glutamate clearance by suppressing expression of glutamine synthetase, EAAT-1 and EAAT2 in astrocytes; impairment of glutamate/GABA-glutamine shuttle represents a leading mechanism of As-induced neurotoxicity (Zhao et al., 2012).

3. Astroglial zink and neurodegeneration

Zinc is the second most abundant trace element in mammalian tissues and is indispensable for normal brain functions (Paoletti et al., 2009). Zinc dyshomeostasis is associated with several neurological disorders including depression, schizophrenia, AD, ALS and aging-related cognitive decline (Levenson and Tassabehji, 2007; Grabrucker et al., 2011; Adlard et al., 2014). Astrocytes can rapidly accumulate zinc through zinc-transporters (ZnT) including Zn-regulated and iron-regulated transporter proteins 14 (ZIP14) and ZnT3, thus, protecting neurones against Zn toxicity (Nolte et al., 2004; Bishop et al., 2010; Sun et al., 2012). Over-accumulation of Zn in the brain can trigger the aggregation of β -amyloid and formation of associated senile plaques, thus, contributing to the AD-type neurodegeneration (Hancock et al., 2014). Chronic exposure to Zn can promote the deposition of β -amyloid and an increase in S100A6 (an acidic $\text{Ca}^{2+}/\text{Zn}^{2+}$ -binding protein) in APP/PS1 transgenic mice; however, exogenous S100A6 is capable of decreasing the aggregation of β -amyloid by buffering/binding Zn in astrocytes and attenuating the AD-related cognitive deficits (Tian et al., 2019).

4. Iron in brain pathology: The role of glia

Iron is the most abundant metal in the brain responsible for normal physiological

functions and developmental processes (Ashraf et al., 2018). The level of iron is gradually increasing with ageing in the substantia nigra, in basal ganglia and cortex (Ramos et al., 2014), albeit the reason for this specific accumulation remains unknown. Excessive accumulation of iron in the brain has been regarded as the major high risk for the neurodegenerative diseases, including AD, PD and ALS (Belaidi and Bush, 2016; Liu et al., 2019; Kwakye et al., 2019; Stephenson et al., 2014).

In physiological conditions, iron contributes to oxygen transportation, mitochondrial respiration, myelin formation, DNA replication and cell signalling (Dev and Babitt, 2017). Homeostatic iron control is fundamental for human health, because both the deficiency and overload of iron are harmful. Iron deficiency is one of the most abundant nutritional deficient diseases. The most common disease is the iron deficiency anaemia, which occurs in infants, adolescents, pregnant women and it presents as secondary symptoms of many clinical conditions, such as gastrectomy and inflammatory bowel disease (Wan et al., 2019). In contrast, iron overload usually emerges in patients with chronic liver or kidney diseases, or results from the iatrogenic treatments including excessive therapeutic supplementation and haemodialysis, or caused by the excessive dietary intake and nutritional supplements (Rostoker and Vaziri, 2017a and 2017b; Lu et al., 2020; Ceylan et al., 2019).

4.1 Iron transport and homeostasis in the brain

Iron from food comes in two forms, the heme iron and non-heme iron, with the latter accounting for 90% of the total iron. Non-heme iron is mainly taken in the brush border of duodenal enterocytes (Zhang Y et al., 2017), the cytochrome b of the cellular membranes of these cells reduces Fe^{3+} to Fe^{2+} , while the latter is transported by plasmalemmal DMT1. The heme iron absorption proceeds through the uptake of the heme carrier protein 1 (HCP-1) (Shayeghi et al., 2005; Krishnamurthy et al., 2007), while the export of the intracellular iron occurs through the ferroportin1 (Fpn1) (Troade et al., 2010). After Fe^{2+} comes into the blood circulation, it is oxidized to Fe^{3+} by the ferroxidases including hephaestin (HEPH) and ceruloplasmin (CP); Fe^{3+} is transported by binding to transferrin (Tf) (Chen et al., 2004; Hellman and Gitlin, 2002). Several pathways translocate iron across the BBB: (i) TF-bound Fe^{3+} by virtue of TFR mainly crosses the luminal membrane of the endothelium; (ii) TF-bound Fe^{3+} can also be transported by transcytosis; (iii) Fe^{2+} is mainly transported by DMT1 localised on the luminal membrane of endothelial cells; (iv) Fpn1 is responsible for the export of iron from endothelial cells to the extracellular space of the brain parenchyma (Jiang et al., 2019; Qian and Ke, 2019). After entering the brain, iron binds to Tf, which is mainly secreted by epithelial cells of the choroid plexus (Leitner & Connor, 2012). Compared with the peripheral tissues, the concentration of non-transferrin-bound iron (NTBI) is higher in the CSF and interstitial fluid (IF), because citrate and ascorbate secreted by astrocytes help to maintain iron in the reduced Fe^{2+} status (Knutson, 2019; Ji and Kosman, 2015). The *glia limitans vascularis* formed by astroglial endfeet covering blood vessels plays a crucial role in

regulating iron homeostasis in the brain through expressing DMT1 to uptake Fe^{2+} (Dringen et al., 2007; Simpson et al., 2015). Early immunohistochemical studies failed to detect TFR or DMT1 in the adult mouse astrocytes (Moos, 1996; Moos & Morgan, 2004). Recently, however, expression of TFR and DMT1 have been demonstrated in astrocytes *in vitro* as well as *in vivo* (Lis et al., 2004; Pelizzoni et al., 2012; Rathore, Redensek, & David, 2012; Urrutia et al., 2013; Zarruk et al., 2015; Xu et al., 2019; Qian et al., 1999; Hoepken et al., 2004). Therefore, astrocytes are able to accumulate Fe^{3+} and Fe^{2+} by the TFR and DMT1 pathways, respectively (Tulpule et al., 2010). In addition, the uptake of NTBI into astrocytes can be mediated by ZIP14 (Bishop et al., 2010).

4.2 Iron overload and neurodegeneration

Patients with PD have higher iron content in the substantia nigra pars compacta as seen by magnetic resonance imaging (MRI) (Ulla et al., 2013; Wieler et al., 2015). Similarly, iron is accumulated in neurones of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced monkey and mouse models of PD (He et al., 2003; Wang et al., 2009; Jiang et al., 2003; Youdim et al., 2003). Increase in iron concentration in dopaminergic neurons aggravates the oxidative stress in which iron reacts with H_2O_2 produced by dopamine metabolism with subsequent generation of $\text{OH}^\cdot$ radicals that can damage proteins, nucleic acids, and membrane phospholipids (Dias et al., 2013; Melis et al., 2013). Furthermore, ROS can induce additional release of iron from mitochondria, thus, further stimulating production of ROS (Ward et al., 2014). This vicious circle and excessive accumulation of ROS are arguably instrumental in instigating cellular death by oxidizing proteins (Melis et al., 2013; Ward et al., 2014). This type of cell death is known as ferroptosis, as the overload with iron causes the overwhelming oxidative damage and the lethal increase in lipid hydroperoxides (Reed and Pellecchia, 2012).

Abnormal iron accumulation is also considered as an early hallmark for AD. Iron elevation in the AD brain was first reported in 1953 (Goodman, 1953), and its association with β -amyloid and neurofibrillary tangles or with ferritin in peripheral glial cells was also documented (Kim et al., 2018; Mandel et al., 2007; Ward et al., 2014). The level of iron in deep grey matter and the neocortex reported by 3T MRI was higher in AD-patients when compared to healthy control subjects (Damulina et al., 2020). Elevated iron in AD-patients is also related to the degree of cognitive impairments (Derry and Kent, 2017). Abnormally increased iron in the brain of AD patients can interact with H_2O_2 to produce highly active hydroxyl radicals, which damage cellular structures (Levi and Tiranti, 2019). In AD patients, TFR is reported to be increased in the hippocampus (Morris et al., 1994), although the level of Fpn is reduced in many cerebral regions (Raha et al., 2013), which may indicate the enhanced uptake of iron which accumulates in neuronal cells. However, the low levels of plasma iron are also reported in AD-patients (Faux et al., 2014; Camaschella, 2013), which may be attributed to the abnormal loading and desaturation of TF (Hare

et al., 2015). Hence, the relationship between iron overload and AD pathology still requires further research.

Through MRI or quantitative susceptibility mapping (QSM), iron accumulation can also be observed in the motor cortex in ALS (Bhattarai et al., 2020; Ignjatovic et al., 2013). Increased ferritin and the decreased TFR were reported in plasma of ALS patients (Mitchell et al., 2010; Qureshi et al., 2008). Similarly, increases in serum iron, ferritin and saturated TF were found in ALS patients (Veyrat-Durebex et al., 2014); serum ferritin is even considered as a biomarker for ALS progression (Yu et al., 2018). In addition, the level of iron is also increased in the CSF of ALS patients (Hozumi et al., 2011), which may translate into iron-induced oxidative stress and ROS generation (Ignjatovic et al., 2012). Iron overload in the brain may stimulate neuroinflammation by activating TNF- α converting enzyme (Lee et al., 2015). Finally, mutations of genes encoding the regulation related proteins of iron, such as homeostatic iron regulator (HFE) and SLC11A2 (encoding DMT1) genes, have been observed in ALS (Guida et al., 2018; Nandar et al., 2013 and 2014; Blasco et al., 2011).

4.3. Iron toxicity and neuroglia

In the CNS, astrocytes accumulate Fe^{2+} through plasmalemmal transporters DMT1 and ZIP14 (Codazzi et al., 2015; Bishop et al., 2010). Astrocytes can also accumulate Fe^{3+} bound to TFR, the expression of which was found in cultured astrocytes (Zarruk et al., 2015) and recently confirmed *in vivo* (Xia et al., 2020). Astrocytes also contribute to maintaining the pool of Fe^{2+} in the brain by secreting acidic interstitial buffers (Hohnholt et al., 2013; Pelizzoni et al., 2013; Ji and Kosman, 2015). Iron is stored as ferritin in astrocytes and is released by Fpn and the ferroxidase CP (Wu et al., 2004); the deficiency of CP can also lead to the iron overload in the brain and neurotoxicity (Jeong and David, 2003).

In iron-induced chronic seizure models, astrocytic EAAT expression is persistently decreased in the hippocampus, whereas the neuroactive androgen steroid dehydroepiandrosterone can exert an antiepileptic action by up-regulating these transporters (Mishra et al., 2013). With ageing, the permeability of the BBB is increased, and iron deposition is also increased in astrocytes from the cortex, hippocampus and basal ganglia (Farrall and Wardlaw, 2009; Block et al., 2007). However, iron overloaded astrocytes may become a trigger of neurotoxicity that contributes to the pathogenesis of age-dependent neurodegeneration, which involves iron-induced oxidative stress and mitochondrial malfunction (Dringen et al., 2007; Schipper et al., 2009). In the frontal cortex, iron dextran increases the glial fibrillary acidic protein (GFAP)-positive astroglial profiles (Liang et al., 2020), which is indicative of reactive astrogliosis (Fig. 1).

As shown in Fig. 2, the iron overload can impair the brain-wide glymphatic system (Liang et al., 2020), responsible for the clearance of waste proteins through a

paravascular pathway (Ilf and Nedergaard, 2013). In the mice model of depression induced by chronic unpredictable mild stress (CUMS), the function of glymphatic system is suppressed by down-regulation of the expression of the astrocytic water channel aquaporin 4 (AQP4) (Xia et al., 2016). Injection of iron dextran further worsens the operation of the glymphatic system and exacerbates the depressive-like behaviours induced by CUMS; in turn, this triggers neuronal apoptosis (Liang et al., 2020). Hence, the iron supplements for major depressive patients should be monitored. Coincidentally, iron oxide nanoparticles (widely applied in biological and medical fields) are also reported to cause toxic damage to human astrocytes (Valdiglesias et al., 2016; Coccini et al., 2017).

Microglial cells, responsible for innate brain immunity, are also involved in iron homeostasis. Microglia are the most efficient in accumulating iron as a cell type in the brain, followed by astrocytes, and then neurons (Bishop et al., 2011). However, this may differ between brain regions (Reinert et al., 2019), because expression of iron transporters varies greatly in neural cells from different brain regions (Rouault, 2013). Astrocytes are well known to regulate the transport of iron to other neural cells (Dringen et al., 2007), and microglia supplies iron to oligodendrocytes to ensure their demand of iron (Zhang et al., 2006). Accumulation of iron in microglia gradually increases with age through the elevation of ferritin (Lopes et al., 2008). Ferritin positive dystrophic microglia are associated with β -amyloid plaques and neurofibrillary tangles (Streit et al. 2014). Aberrant iron homeostasis increases the release of proinflammatory cytokines from microglia *in vitro* (Wang et al. 2013). Neuromelanin, a protein that stores iron in neurones can be phagocytosed by microglia, which can increase production of proinflammatory cytokines and ROS, thus, exacerbating neurodegeneration (Rathnasamy et al. 2013). Accumulation of iron in microglia is consistently observed in the neurodegeneration-prone brain regions, providing a high correlation between the pathological phenomena of neurodegeneration and the increase of iron in microglia (Andersen et al., 2014).

Conclusion and future directions

Rising environmental contamination and increased presence of heavy metals in general life along with related neurotoxicity are gaining increasing attention. Astrocytes are key protectors of neurones in the CNS, but under the exposure to excessive heavy metals, astrocytes may become the main targets. Heavy metals, such as Mn, Pb, Hg and iron, all can destroy the integrity of nervous tissue and affect glial-neuronal interactions. In particular, heavy metals severely disrupt glutamate homeostasis through affecting expression and efficacy of glutamate/GABA-glutamine shuttle at multiple levels including suppression of glutamine synthetase activity and down-regulation of plasmalemmal glutamate transporters. Heavy metal-induced glutamate excitotoxicity evokes pathological Ca^{2+} signalling and damages intracellular Ca^{2+} homeostasis, triggers oxidative stress, destroys mitochondria, and instigates cell death. The neurotoxicity caused by heavy metals in astrocytes can play

a deteriorative role in facilitating neurodegenerative diseases. Among these heavy metals, some are key trace elements for physiological and developmental processes, like iron, so its homoeostasis is essential for proper operation of the CNS. Iron overload in neurodegenerative disorders is widely reported, while MRI images support the accumulation of iron in brains of patients suffering from AD, PD and ALS. Iron-mediated neurotoxicity is also associated with reactive astrogliosis and impairments of the lymphatic system, which may also contribute to the progression of neurodegenerative diseases.

Future research need to consider several issues:: (i) monitoring of heavy metals in the CNS should receive more attention, especially of the iatrogenic origin; (ii) the reasons for the metals deposition in specific brain regions are little known, although these depositions may directly contribute to the occurrence of neurodegenerative diseases, such as PD; (iii) the ways to effectively reduce the neurotoxicity induced by heavy metals using antioxidants, anti-inflammation agents, and/or by (epi)genetic modulation; (iv) understanding the relationship between the accumulation of heavy metal(s) and brain ageing and whether the effective clearance of excessive metal can slow the ageing process and improve the cognitive longevity.

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Figure Legends

Figure 1. 3D-images of GFAP labelled astrocytes in frontal cortex.

After treatment with dextran (control) or 2 mg/kg/day iron dextran for 6 days, 3D-images of GFAP residing in astrocytes were taken in the mouse frontal cortex indicating a development of reactive astrogliosis.

Figure 2. Excess iron aggravates malfunction of glymphatic system in chronic unpredictable mild stress-treated mice.

(**A, B**) Mice were pre-treated with or without chronic unpredictable mild stress (CUMS) for 6 weeks; in the last week the mice were randomly separated to be injected with dextran or iron dextran for 6 days. The fluorescence tracer (OA555, 45kDa) was injected intracisternally. (**A**) Representative images indicated the fluorescence tracer penetration into the brain; OA555 (red) and DAPI (blue; cell nuclei label) were stained simultaneously, in anterior and posterior brain slices. Scale bar, 1 mm. (**B**) Thirty minutes after injection, the animals were perfusion fixed and the whole-slice fluorescence was calculated. The fluorescence intensities of OA555 normalised to the intensity of the control group were assessed. Scale bar, 50 μ m. Data are presented as mean $\pm$ SEM, n = 6. *p < 0.05, statistically significant difference compared to the control group. **p < 0.05, statistically significant difference compared to any other group. (Reproduced from Liang et al., 2020 with permission).

Control

Iron

Fig.1

B

Fig. 2