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REVIEW

# Role of Astrocytes in Manganese Neurotoxicity Revisited

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## Abstract

Manganese (Mn) overexposure is a public health concern due to its widespread industrial usage and the risk for environmental contamination. The clinical symptoms of Mn neurotoxicity, or manganism, share several pathological features of Parkinson's disease (PD). Biologically, Mn is an essential trace element, and Mn in the brain is preferentially localized in astrocytes. This review summarizes the role of astrocytes in Mn-induced neurotoxicity, specifically on the role of neurotransmitter recycling, neuroinflammation, and genetics. Mn overexposure can dysregulate astrocytic cycling of glutamine (Gln) and glutamate (Glu), which is the basis for Mn-induced excitotoxic neuronal injury. In addition, reactive astrocytes are important mediators of Mn-induced neuronal damage by potentiating neuroinflammation. Genetic studies, including those with *Caenorhabditis elegans* (*C. elegans*) have uncovered several genes associated with Mn neurotoxicity. Though we have yet to fully understand the role of astrocytes in the pathologic changes characteristic of manganism, significant strides have been made over the last two decades in deciphering the role of astrocytes in Mn-induced neurotoxicity and neurodegeneration.

**Keywords** Manganese · Neurotoxicity · Astrocyte · Glutamine · Glutamate

## Introduction

Manganese (Mn) is an essential trace element, serving as an enzymatic cofactor, yet, Mn deficiency is uncommon. Human overexposure to Mn occurs in occupational settings and/or contaminated areas [1, 2]. A variety of anthopogenic sources of Mn increases the risk for human overexposure. Mn is widely used for multiple industrial purposes, including iron and stainless steel production, formation of aluminum alloys, and synthesis of Mn oxide electrodes, antiknock additive, and fungicides [4, 5], to name a few. In the general population, exposure to Mn occurs largely from consumption of water containing high levels of the metal [6], from infant formulas [7] and from atmospheric Mn due to combustion of methylcyclopentadienyl Mn tricarbonyl, a petrol antiknock additive [8]. In adult humans, approximately 3–5% of ingested Mn is absorbed in the intestine; however, molecular mechanisms of Mn uptake are not well understood [9]. The developing brain is susceptible to Mn toxicity [3]. Dietary Mn crosses the blood–brain barrier (BBB) and/or the choroid plexus to deposit in the brain [10–12] via its transporters, particularly divalent metal transporter-1 (DMT-1) and transferrin receptor (TfR) [13]. Other transporters include purinoceptors, choline transporters, and transient receptor potential melastatin 7 (TRPM7) [14]. Mn is predominantly transported across the BBB as a free ion, but it has yet to be determined which valent species is transported under physiologic conditions [15]. Although the transporters used to shuttle Mn across the BBB may vary by physiological conditions, it is proposed that $Mn^{3+}$ is transported by TfR, while $Mn^{2+}$ is transported by DMT-1 [11, 14]. For an extensive review on Mn transport across BBB, please see [14].

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In humans, elevated blood levels of Mn can be attributed to environmental exposure or failed excretion of Mn due to hepatic dysfunction. In a 1-year follow up study of 51 cirrhosis patients, 11 (21.6%) patients developed Parkinsonian symptoms or hepatocerebral degeneration [16]. Once it reaches the brain, Mn primarily deposits in the basal ganglia. [17]. High signals of T1-weighted MRI scans were found in the globus pallidus in these biliary atresia patients having elevated levels (7–72 μg/ml) of Mn in blood serum. Among them, one female with 7 μg/ml of Mn in blood serum experienced depressive symptoms and dyskinesia. It was reported that this patient has recovered after levodopa (L-dopa) treatment [17]. Using a similar MRI scan technique to detect Mn signals in the brain of a welder who had 1.76 ug/ml blood serum Mn, high intensity signals were found in the substantia nigra, subthalamic region, and globus pallidus [18]. The welder developed typical parkinsonian symptoms, including bradykinesia, cogwheel rigidity, and slight resting tremor, as well as short-term and long-term memory deficits [18]. In contrast to adults, infants and developing children are particularly at risk for toxicity following Mn ingestion. High levels of Mn in trace element-supplemented neonatal parenteral nutrition are a potential risk for infants [19]. An MRI imaging study found that 4-week supplementation of neonatal parenteral nutrition with Mn was associated with high Mn signals in the globus pallidus and putamen in infants [19].

Mn is preferentially localized in astrocytes in the brain. An in vitro study revealed that uptake of $Mn^{2+}$ by astrocytes is dependent on transferrin and DMT1 [20]. The propensity of Mn accumulation in astrocyte is likely related to its biological roles, but it is unclear whether intracellular level of Mn is governed by its biologic needs. A Mn-dependent enzyme, glutamine synthetase (GS), is predominantly expressed in astrocyte [21, 22]. An in vitro study demonstrated that Mn is also necessary for regulating astrocyte morphology [23]. In addition, Mn is an important activator for many enzymatic proteins, such as Mn- superoxide dismutase (Mn-SOD) in mitochondria [24], arginase I, which is localized primarily in the cytoplasm of hepatocytes [25], and arginase II, which is located in mitochondria, with the highest abundance in the kidney [26], to name a few. It is noteworthy that both arginase I and arginase II can be detected in neurons, but not in glial cells in mouse brain [27]. In the prodromal Huntington's disease (HD) mouse model, Mn supplementation restored arginase dependent urea cycle homeostasis in the striatum [28].

One of the characteristics of Mn as a nutritional metal is that the estimated safe window of Mn for physiological function is in a narrow range, from 20.0 to 52.8 μM Mn [29]. Low level of Mn pretreatment can reduce the rate, area, and amplitude of mechanically induced $Ca^{2+}$ waves in astrocyte [30]. The reduced $Ca^{2+}$ wave could be explained by the sequestration of $Ca^{2+}$ within the mitochondria and diminished available pool of releasable $Ca^{2+}$ in endoplasmic reticulum (ER) [30]. Recently, it was hypothesized that the level of Mn for maximum biologic benefits may also exert toxic effects [31]. In this regard, the links between Mn nutritional roles and its harmful effects should be considered in future research. As other nutritional metals, cellular Mn must be tightly regulated to maintain at an optimized level. Indeed, it has shown that mutation of SLC30A10, one of cellular membrane localized divalent efflux transporters, leads to cellular mislocation of this protein, compromised Mn metabolism, and Parkinsonism [32, 33].

## Metabolic Coupling Between Astrocytes and Neurons

The metabolism of astrocytes is tightly coupled with the activity of neurons [34]. Metabolic interaction between neurons and astrocytes is critical for maintaining homeostasis of neurotransmitters: glutamate (Glu) and γ-aminobutyric acid (GABA) [35]. This interaction, known as the glutamine (Gln)/Glu cycle (GGC), involves Gln synthesis by astrocyte-specific enzyme GS and the subsequent release of Gln from astrocytes to the extracellular space [36]. The released Gln is then taken up by neurons and metabolized to Glu by the neuron-enriched enzyme glutaminase. Glu released from neurons is transported to astrocytes, and amidated to Gln by GS, completing the cycle (Fig. 1). In addition to supplying the neurotransmitter pool of Glu, GGC is also involved in the homeostasis of GABA [37]. The biosynthesis of GABA strictly relies on the adequate supply of Glu within GABAergic neurons and on the subsequent conversion of Glu to GABA by Glu decarboxylase (GAD) [38, 39]. Thus, the mechanisms responsible for metabolism and transfer of Glu and Gln in astrocytes and neurons are fundamental to brain physiology.

The homeostasis of Glu in synaptic cleft and integrity of Glu-mediated neurtransmission is maintained by multiple mechanisms [35]. Specifically, Glu in presynaptic neuron is stored in synaptic vesicles until a nerve impulse triggers the release of Glu into the synaptic cleft, where it binds to Glu receptors on the postsynaptic membrane [40]. After that, Glu is removed from the synapses by astrocytes through sodium-dependent transporters localized on astrocytic cell membrane, namely GLAST (Glu-aspartate transporter; or EAAT2/SLC1A3) and GLT1 (Glu transporter 1; or EAAT2/SLC1A2) [41]. Transport of Gln across the membranes of neural cells is mediated by multiple transport systems, with overlapping substrate specificity and different substrate affinity and cellular distribution [42]. Among these systems, the sodium-dependent systems N, ASC, and A play dominant role in Gln turnover. The bi-directional system

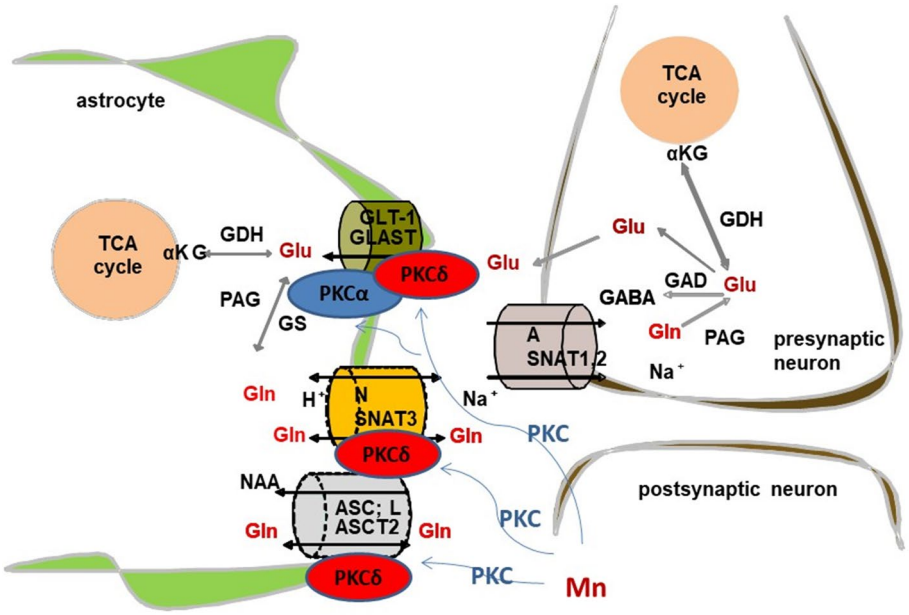


**Fig. 1** Mn deregulates Gln and Glu transporters expression and function by PKC pathway activation. Glu released from synaptic terminals is taken up by surrounding astrocytes via Glu transporters and converted to Gln via the reaction mediated by GS. A proportion of Gln is released into the extracellular space by Gln carriers, with a predominant role of system N. In addition to system N, release of Gln from astrocytes is mediated by transporters belonging to systems L and ASC. Extracellular Gln is taken up into GABAergic and glutamatergic neurons by the unidirectional system A transporters. Once in neurons, Gln serves as a substrate for the mitochondrial enzyme, PAG for the synthesis of Glu, which supply neurotransmission pool of Glu, or can be converted to GABA by GAD or to αKG by GDH. Excessive levels of Mn deregulates processes related to the Gln and Glu transporter expression and function by PKC pathway activation. *αKG* αketoglutaric acid, *GABA* γ-aminobutyric acid, *GAD* glutamate decarboxylase, *GDH* glutamate dehydrogenase, *Gln* glutamine, *Glu* glutamate, *NAA* neutral amino acids, *PAG* phosphate activated glutaminase, *PKCα* protein kinase C alpha, *PKCδ* protein kinase C delta, *TCA* tricarboxylic acid

N transporters SNAT3 (sodium coupled neutral amino acid transporter 3; or SLC38A3) and SNAT5 (sodium coupled neutral amino acid transporter 5; or SLC38A5) are present exclusively in astrocytes [43], while SNAT3 is believed to play a major role in the release of Gln. In addition to system N, release of Gln from astrocytes is mediated by other transport systems, such as system ASC (ASCT2, alanine-serine-cysteine transporter 2; or SLC1A5) and sodium-independent system L (LAT2, L-type AA transporter 2; or SLC7A5) [44, 45]. The unidirectional system A transporter SNAT1 (SLC38A1) is predominately responsible for Gln transport to neurons [46]. Abnormal expression and function of Gln carriers has been reported in several neuropathological conditions such as neurotoxicity, CNS carcinogenesis and neurodegeneration [47].

## Dysregulation of GGC in Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease (PD), and Alzheimer's Disease (AD)

### ALS

ALS is a neurodegenerative disease characterized by the progressive degradation of motor neurons in the brain and spinal cord [48]. The mechanisms of ALS are not well understood, but studies demonstrate that the disruption of astrocyte-neuronal integrity and subsequent GGC dysregulation is closely related to ALS pathogenesis [49]. Glu-mediated excitotoxicity in the motor cortex has been hypothesized as a pathophysiological marker of ALS, as patients with ALS exhibit elevated Glu levels in their cerebrospinal fluid [50], and knockdown of metabotropic Glu receptors delayed the onset of disease and prolonged survival in an ALS mouse model [51, 52]. In accordance with these findings, a recent study demonstrated that treatment with riluzole, which blocks Glu release and postsynaptic receptor activation, prolonged survival in patients with advanced ALS in clinical trials, though the mechanism of action is not well understood [53]. Similarly, treatment with methionine

sulfoximine (MSO), a GS inhibitor, prolonged survival in a $SOD1^{G93A}$ mouse model of familial ALS, suggesting that GGC dysregulation contributes to ALS [54].

### PD

PD is an age-related neurodegenerative disorder caused by the loss of dopaminergic neurons within the substantia nigra pars compacta. Both environmental and genetic factors may cause PD. A plethora of hypothesis have been proposed to be the underlying reasons for dopaminergic neurodegeneration associated with PD, such as dysfunction of the ubiquitin–proteasome pathway, mitochondrial dysfunction, oxidative stress, ATP depletion and the activation of cell death pathways [55]. It is likely that environmental factors play a prominent role in the development of PD [56], however the key molecular events that provoke neurodegeneration are still unclear [55]. Studies have demonstrated that astrocyte dysfunction contributes to PD progression that might involve dysregulation of the GGC in the process [57]. Excitotoxicity describes the pathological process through which neuronal cell death is induced after excessive stimulation of glutamatergic receptors by Glu [57]. Accordingly, Glu also contributes to excitotoxic-neuronal death which could exacerbate nigrostriatal neurodegeneration in PD [58, 59].

Though no neuroprotective therapies pertinent to PD causes are currently available, the discoveries from PD models have elucidated some important aspects of the molecular cascade of cell death in dopaminergic neurons [60]. Nuerotoxin-based PD model has illustrated the selective vulnerability of dopaminergic neurons to chemicals including 6-hydroxydopamine/6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/MTPT, rotenone and paraquat [60]. The neurotoxicant MPTP as a PD animal model increased Glu levels in the striatum of mice as well as enzymes that are involved in the GGC such as GS in astrocytes and glutaminase in presynaptic neurons in the mouse brain [61]. These results suggest that increased conversion of Gln to Glu contribute to abnormally high levels of Glu in the synaptic clefts [62]. Studies have also shown that MPTP reduced the reuptake of Glu in cultured astrocytes [63], indicating that impaired Glu reuptake may lead to excess Glu in the synaptic clefts and consequential excitotoxicity. These findings corroborates with the hypothesis that dysregulation of the GGC is associated with chemically-induced PD [57]. In agreement with this, the GGC is a potential early target for Mn neurotoxicity [35] (please see below for the mechanisms of Mn-induced dysregulation of the GGC). Importantly, Mn neurotoxicity shares multiple common mechanisms with the above mentioned PD-mimicking neurotoxins. Occupational exposure to Mn is associated with increased risk of PD [56]. The strongest correlation between any type of environmental exposure and PD is noted in human studies of non-occupational Mn exposure cohorts [56].

### AD

Recent evidence suggests that astrocytes play a critical role in brain homeostasis and the progression of various neurological disorders including Alzheimer's disease (AD) [64]. Studies showed that higher Glu and Gln levels in the cerebrospinal fluid of AD patients are significantly correlated with increased levels of AD biomarkers, β-amyloid and tau, compared to healthy controls [65], suggesting that excess levels of Glu may contribute to the formation of β-amyloid and tau prior to the onset of AD. These studies bring important information on the link between Glu dyshomeostasis and AD that could offer a unique and robust approach in exploring potential biomarkers and treatment for AD.

In AD patients, GS is greatly reduced compared to healthy aging population [66–68]. Studies indicate that reduction of GS in AD brains may be caused by translational or post-translational aberrations of the protein [67, 68]. GS is highly sensitive to oxidative stress which could lead to proteolysis of GS and its inactivation [69]. A triple-transgenic mouse model of AD (3xTg-AD) also showed a gradual decrease in GS-positive astrocytes in mice [70], suggesting an interaction between Glu dysregulation, β-amyloid and tau in AD pathogenesis. These studies suggest that reduction of GS, either by its quantity or functionality, lead to dysregulation of GGC which contribute to AD pathology.

Glu transport is also an integral part of GGC that reuptakes synaptic Glu immediately after dissociating from post-synaptic Glu receptors. Studies showed that astrocytic Glu transporters are reduced in AD patients [71–73]. Reduced Glu transporters correlate with increased Glu levels and neuronal death [72], indicating that impaired Glu uptake could cause a cascade of events leading to neurodegeneration and memory impairment. Reduction of GLT-1 and Glu uptake in the forebrain of a transgenic mouse model of AD ($APP_{Sw,Ind}$) is correlated with memory impairment and increased mortality [74]. In addition, restoration of GLT-1 expression and function attenuated memory and learning deficits of $APP_{Sw,Ind}$ mice [74], suggesting that Glu transport and homeostasis is critical in AD.

## Mn Disrupts Astrocytic-Neuronal Integrity

Several studies have established the propensity of Mn to affect major components of the GGC, leading to significant decline of Glu transporter functions. Mn treatment (500 μM, 6 h) attenuated Glu transportation in GLAST and GLT-1 overexpressed CHO-K1 cells (Chinese hamster ovary cell line) [75]. In non-human primate brain, long-term airborne

Mn exposure (1.5 mg Mn/$m^3$, 33 days) was associated with downregulation of GLAST and GLT-1 transporter expression [76].

Many factors have been identified to influence the onset and progression of AD which includes a combination of genetic, lifestyle and environmental factors [77]. Studies have shown that environmental toxicants including Mn contribute to cognitive and memory deficits in humans and in vivo animal models [78–80]. While studies showed Mn toxicity caused cognitive and memory deficits in mice [80], the underlying mechanism of Mn toxicity and decline in memory function needs further investigation. Of particular interest, Mn has shown to disrupt the GGC at multiple levels including Glu metabolism and transport. Mn as a cofactor of GS can directly influence Glu cycling [21, 22]. In addition, high levels of Mn decreased GS activity and expression in rat primary astrocytes [81], suggesting the buildup of excess Glu that may contribute to excitotoxicity. Studies have also shown that Mn deregulates astrocytic Glu transporters EAAT1/GLAST and EAAT2/GLT-1 in both in vitro and in vivo settings resulting in behavior and motor deficits [82, 83]. Attenuation of Mn-induced reduction of GLAST and GLT-1 with treatment with histone deacetylase inhibitors, 17β-estradiol (estrogen) and tamoxifen restored behavior and motor function of mice [83, 84]. These findings provide evidence of a potential link between Mn toxicity and AD pathology through inhibition of Glu transporter function or GS activity resulting in impaired GGC, disrupted synaptic homeostasis and neuronal dysfunction.

## Mechanisms of Mn-Induced Decrease of Glu Transporters

### Aberrant PKC Signaling

Several studies have proposed mechanisms of Mn-induced downregulation of Glu transporters expression and function. For example, inhibition of lysosomal-dependent proteolysis pathways blocked Mn-induced disruption of Glu transporters activity and expression [85, 86]. PKC stimulation by α-phorbol 12-myristate (PMA) significantly decreased astrocytic Glu uptake, whereas treatment with the general PKC inhibitor bisindolylmaleimide II (BIS II), specific PKCδ isoform inhibitor rottlerin (ROT) or specific PKCα isoform inhibitor Gö6976 (Gö) reversed Mn-induced downregulation of Glu uptake in primary cultures of astrocytes [37]. Similar to the uptake study, Mn-induced downregulation of GLT-1 at protein level was reversed by BIS II, ROT, and Gö6976 inhibitors, while decreases in GLAST protein levels were reversed only in the presence of PKCα isoform inhibitor [37]. Furthermore, immunoprecipitation studies demonstrated GLT-1 co-expression with PKCδ and PKCα isoforms, and increased expression of GLT-1 is specifically associated with PKCδ upon exposure of astrocyte to Mn.

### Activation of Transcription Factors Yin Yang 1 (YY1) and NF-κB

Studies of the Mn-induced inhibition of Glu transporter expression and function have revealed the prominent role of YY1. YY1 overexpression significantly decreased GLT1 promoter activity. The inhibitory effect of Mn on GLT-1 promoter activity was reversed in the YY1 mutant construct of GLT-1 in cultured astrocytes. Further studies revealed that an increase in YY1 expression upon Mn exposure was induced by NF-κB activation, which also enhanced YY1 binding to the GLT-1 promoter region, and histone deacetylases (HDACs) were also recruited as epigenetic cofactors for the inhibitory effect of YY1 on GLT-1 [87]. YY1 was also found as a critical mediator in the inhibitory effects of Mn on GLAST, while knockdown of YY1 reversed the Mn-induced decreases in GLAST promoter activity and mRNA/protein levels in rat primary astrocytes and human astrocytic H4 cells [87]. Analogous to GLT-1 regulation by Mn, studies revealed evidence for YY1 and HDACs co-repression activity that affect GLAST expression [87]. Further studies identified consensus sequences of NF-κB (two putative binding sites) and YY1 (nine putative binding sites) in the GLAST promoter region in human astrocytes. NF-κB p65 subunit was found to positively regulate GLAST transcription, while YY1 negatively regulates GLAST transcription. The study demonstrated that Mn not only activated NF-κB, but also affected the nuclear interaction between NF-κB p65 and YY1 that causes repression of GLAST transcription in human H4 astrocytes [88].

## Mechanisms of Mn-Induced Disruption of Gln Transporters

### Dysregulation of Ubiquitin-Proteolysis Process

Unlike Glu, the transport of Gln within the GGC is more heterogeneous and strictly depends on astrocyte-neuron dialogue, where efflux of Gln from astrocytes must be met by influx of amino acid in neurons. Mn toxicity is directly related to the dysfunction of both of these GGC-associated transporting processes. It was shown that pretreatment of neonatal rat cortical primary astrocytes with Mn (100 μM, 1 min) inhibits the initial net uptake of Gln in a concentration-dependent manner [89]. Detail activity analysis of astrocytic systems transporting Gln in the presence of Mn revealed significant decrease in Gln uptake by two principal Gln-transporting systems, namely N and ASC. Moreover, Mn decreased Gln efflux from astrocytes via systems N, ASC and L. Mn also decreased mRNAs levels of Gln

transporters SNAT3, SNAT2 and LAT2 in astrocytes [90]. Notably, a recent study revealed that Gln transporter SNAT3 displayed the highest sensitivity to Mn, which was manifested as SNAT3 protein degradation in response to a relatively short Mn exposure (500 μM, 4 h) [90].

It has been reported that there is a link between Mn-mediated downregulation of SNAT3 and activation of proteolytic processes preceded by ubiquitination in the primary cultured astrocytes [85]. Study revealed that Mn increased the free ubiquitin levels followed by general protein hyperubiquitination in astrocytes [85]. Mn induced interaction between SNAT3 and the ubiquitin ligase Nedd4-2, which is known to be involved in the internalization and degradation of plasma membrane channels and transporters [85, 91]. Taken together with the observation that Mn increases expression of Nedd4-2 and that Mn-dependent degradation of SNAT3 is reversed by proteasome and lysosome inhibitors, these studies suggest specific regulation of SNAT3 transporter in Mn toxicity.

#### Aberrant PKC Signaling

Similar mechanistic evidence such as PKC signaling augmentation upon Mn-induced downregulation of Glu transportation was also reported for the Gln transporting systems. One such study revealed that PKC inhibition reverses the Mn-dependent decrease in astrocytic Gln uptake, while PKC stimulation downregulates Gln uptake systems ASC and system N and decreases levels of ASCT2 and SNAT3 in cell lysates and in plasma membranes [92]. Exposure to Mn increased binding of PKCδ to ASCT2 and SNAT3, suggesting a prominent role for PKCδ in Mn-mediated disruption of Gln turnover. Both SNAT3 and ASCT2 transporters were found to contain conserved putative PKC phosphorylation sites in humans, rats and mice, which could explain their sensitivity to changes in PKC pathways that are associated with Mn toxicity [93, 94].

## Mn-Induced Dysregulation of the GGC: Implications for Astrocytic-Neuronal Integrity

The Gln transporting system as well as glutamatergic system sustains vulnerable points in the reaction between astrocytes and neurons in Mn toxicity, where astrocytes may fail to support neurons by Gln supplementation, Glu/Gln homeostasis regulation and maintainance of proper Glu extracellular level. Below, we detail the mechanisms and molecular events underlying Mn-mediated disturbance in astrocytic–neuronal integrity, which may have promising implications for the development of future treatment strategies for brain pathology associated with Mn toxicity.

### Astrogliosis, or Reactive Astrocyte Mediates Mn Neurotoxicity

Astrogliosis, or the activation of reactive astrocytes, refers to the functional and structural changes in astrocytes responding to CNS damage and disease [95]. Recent advances show that astrogliosis is a heterogeneous process involving specific cellular and molecular signatures corresponding to different forms of CNS injuries, such as neuroinflammation [95]. In this regard, astrocytes are important mediators of Mn neurotoxicity. Mn treatment potentiated LPS-induced astrocyte inflammation response by increasing mitochondrial reactive oxygen species (ROS) [96]. Treating astrocytes with Mn (50 μM) exaggerated IFN-γ and TNF-α induced elevation of NO and nitric oxide synthase 2 (NOS2) [97].

In an astrocyte and PC12 co-culture system that hindered the direct interaction of the two cells, activation of astrocytes by treatment with both Mn and IFN-γ/TNF-α induced activation of caspases and apparent morphology changes of differentiated PC12 cells, which is believed to be mediated by NF-κB -regulated and partially NO -mediated inflammatory response, however, either Mn or IFN-γ/TNF-α treatment alone did not cause apparent damage to PC12 cells [97]. C57B1/6 mice exposed to Mn (100 ppm, 8 weeks) by intragastric gavage developed typical neurodegenerative movement disorder and pathologic damage, such as astrocyte activation, hypertrophy of endothelial cells, loss of vascular integrity, and appearance of apoptotic cholinergic interneurons but not dopaminergic neurons in areas proximity to capillaries in both the pallidum and striatum [98]. Microglia, a residing macrophage cells in the brain, functions as immunity cells to prevent potential damage caused by either foreign agents or cellular debris. Deregulated activity of microglia is the hallmark of the neurodegenerative diseases and play potential role in the development of Mn-induced neurotoxicity. Indeed, it was shown that co-culture with microglia potentiated Mn induced inflammatory response in astrocyte and neuronal cell death [99]. For an extensive review of neuroinflammation in Mn neurotoxicity, please see [100].

### PD genes in Maintaining Astrocyte Function Play Roles in Mn Neurotoxicity

The genes associated with PD pathogenesis have important biological functions in astrocyte as well, and their roles in Mn-mediated astrocytic damage and neurotoxicity are emerging [101]. These genes include *PARK7*, *SNCA*, *PLA2G6*, *ATP13A2*, *LRRK2*, *GBA*, *PINK1*, and *PARK2* [102]. Mutations in several of these genes may contribute to Mn-induced, astrocyte-mediated neurotoxicity. Deficiency in ATP13A2, a lysosomal type 5 ATPase protein, has been shown to impair lysosomal function and contribute

mitochondrial dysfunction [103]. *ATP13A2*-KO astrocytes exhibited deregulated expression of inflammatory cytokines [104]. *Atp13a2*-deficiency mice exhibited increased sensitivity to Mn toxicity (5 ppm, 45 days), such as enhanced sensorimotor function and increased insoluble alpha-synuclein [105]. DJ-1 is a product of *PARK7*, which plays a role in the assembly of lipid rafts and impacts membrane receptor trafficking, endocytosis, and signal transduction [106]. It has been shown that the DJ-1 expression is higher in astrocyte than in neurons in human [107]. Previous study showed that Mn exposure caused decreased expression of DJ-1 in astrocyte [108]. In *Caenorhabditis elegans* (*C. elegans*), one of DJ-1 homologues, *djr-1.2* protects against Mn toxicity [109].

*C. elegans* is powerful model organism that is being used to investigate conserved biologic functions of genes and dissect genetic roles in the pathogenesis of human diseases. The genomes of *C. elegans* contain more than 20,000 protein coding genes, and many of them are shared with human. Disease models such as neurodegeneration [110, 111], cancer [112], psychiatry diseases [113] have been successfully established in *C. elegans*, and have yielded fruitful insights into novel mechanisms of the diseases. Recently, it was postulated that as mammalian glial cells, *C. elegans*' glial cell provide important functional and nutritional support for adjacent neurons [114, 115]. Metals that are believed to be the environmental factors for the neurodegenerative diseases, such as Mn, have been investigated in *C. elegans* on their toxic mechanism. Hence, combing with techniques of genetic modulation, many hypotheses will be relatively easily tested in *C. elegans* mutant strains, especially on gene and environment interaction. Future research on glia cells and Mn toxicity in *C. elegans* will shed new light on this metal as well as better understanding and management of neurodegenerative diseases.

## Future Directions

Excessive Mn exposure and its neurotoxicity have drawn recent attention. Identifying the cellular and molecular targets of Mn is key to understanding its toxicity and developing prevention strategies. Astrocytes are the most abundant glia cells in the CNS, and play essential roles in brain homeostasis. Upon exposure to elevated Mn, astrocytes may fail to support neurons, compromising Gln supplementation, Glu/Gln homeostasis regulation and the maintenance of proper extracellular Glu levels. Molecular events underlying Mn-mediated disturbances in astrocytic–neuronal integrity may have promising implications for the development of future treatment strategies for brain pathology associated with Mn toxicity. Furthermore, glia-derived inflammatory mediators are contributing factors in Mn-induced neuronal cell injury, and reactive astrocytes can potentiate Mn neurotoxicity by triggering inflammatory responses. Lastly, PD genes associated with Mn toxicity play an important role in maintaining astrocyte function.

Important knowledge gaps that await further investigation clearly exist. First, one of the major targets of Mn is mitochondria, but we know little about how Mn transport is regulated across organelles in specific neuronal cells. Secondly, as the physiological level of Mn is maintained in a relatively narrow range [29], what are the relationships between toxicity induced by elevated levels of Mn and its biologic roles? Using cell-specific RNA-seq techniques, comparative studies on the biologic functions of Mn in different type of neuronal cells may increase our understanding of this metal and its selective toxicity. Third, cell specific genetic modulation in model animals, such as *C. elegans*, may further help us to identify cellular and genetic targets of Mn toxicity, as well as genetics of Mn metabolism. The later will be profitable, in that several transporters for Mn have already been postulated in studies of inherited disorders of Mn metabolism [116].

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Kwakye GF, Paoliello MM, Mukhopadhyay S, Bowman AB, Aschner M (2015) Manganese-Induced parkinsonism and Parkinson's disease: shared and distinguishable features. Int J Environ Res Public Health 12(7):7519–7540. https://doi.org/10.3390/ijerph120707519
2. Lucchini R, Placidi D, Cagna G, Fedrighi C, Oppini M, Peli M, Zoni S (2017) Manganese and developmental neurotoxicity. Adv Neurobiol 18:13–34. https://doi.org/10.1007/978-3-319-60189-2_2
3. Bjorklund G, Chartrand MS, Aaseth J (2017) Manganese exposure and neurotoxic effects in children. Environ Res 155:380–384. https://doi.org/10.1016/j.envres.2017.03.003
4. Blanc PD (2018) The early history of manganese and the recognition of its neurotoxicity, 1837–1936. Neurotoxicology 64:5–11. https://doi.org/10.1016/j.neuro.2017.04.006
5. Aschner M, Lukey B, Tremblay A (2006) The manganese health research program (MHRP): status report and future research needs and directions. Neurotoxicology 27(5):733–736. https://doi.org/10.1016/j.neuro.2005.10.005
6. Wasserman GA, Liu XH, Parvez F, Ahsan H, Levy D, Factor-Litvak P, Kline J, van Geen A, Slavkovich V, Lolacono NJ, Cheng ZQ, Zheng Y, Graziano JH (2006) Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Persp 114(1):124–129. https://doi.org/10.1289/ehp.8030

7. Krachler M, Rossipal E (2000) Concentrations of trace elements in extensively hydrolysed infant formulae and their estimated daily intakes. Ann Nutr Metab 44(2):68–74. https://doi.org/10.1159/000012823
8. Finkelstein MM, Jerrett M (2007) A study of the relationships between Parkinson's disease and markers of traffic-derived and environmental manganese air pollution in two Canadian cities. Environ Res 104(3):420–432. https://doi.org/10.1016/j.envres.2007.03.002
9. Horning KJ, Caito SW, Tipps KG, Bowman AB, Aschner M (2015) Manganese is essential for neuronal health. Annu Rev Nutr 35:71–108. https://doi.org/10.1146/annurev-nutr-071714-034419
10. Murphy VA, Wadhwani KC, Smith QR, Rapoport SI (1991) Saturable transport of manganese(Ii) across the rat-blood brain barrier. J Neurochem 57(3):948–954. https://doi.org/10.1111/j.1471-4159.1991.tb08242.x
11. Rabin O, Hegedus L, Bourre JM, Smith QR (1993) Rapid brain uptake of manganese(II) across the blood-brain barrier. J Neurochem 61(2):509–517
12. Zheng W, Perry DF, Nelson DL, Aposhian HV (1991) Choroid plexus protects cerebrospinal fluid against toxic metals. FASEB J 5(8):2188–2193
13. Fitsanakis VA, Piccola G, Aschner JL, Aschner M (2006) Characteristics of manganese (Mn) transport in rat brain endothelial (RBE4) cells, an in vitro model of the blood-brain barrier. Neurotoxicology 27(1):60–70. https://doi.org/10.1016/j.neuro.2005.06.004
14. Fitsanakis VA, Piccola G, dos Santos APM, Aschner JL, Aschner M (2007) Putative proteins involved in manganese transport across the blood-brain barrier. Hum Exp Toxicol 26(4):295–302. https://doi.org/10.1177/0960327107070496
15. Williams M, Todd GD, Roney N, Crawford J, Coles C, McClure PR, Garey JD, Zaccaria K, Citra M (2012) Toxicological profile for manganese. Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles, Atlanta
16. Burkhard PR, Delavelle J, Du Pasquier R, Spahr L (2003) Chronic parkinsonism associated with cirrhosis: a distinct subset of acquired hepatocerebral degeneration. Arch Neurol 60(4):521–528. https://doi.org/10.1001/archneur.60.4.521
17. Ikeda S, Yamaguchi Y, Sera Y, Ohshiro H, Uchino S, Yamashita Y, Ogawa M (2000) Manganese deposition in the globus pallidus in patients with biliary atresia. Transplantation 69(11):2339–2343
18. da Silva CJ, da Rocha AJ, Mendes MF, Braga AP, Jeronymo S (2008) Brain manganese deposition depicted by magnetic resonance imaging in a welder. Arch Neurol 65(7):983. https://doi.org/10.1001/archneur.65.7.983
19. Aschner JL, Anderson A, Slaughter JC, Aschner M, Steele S, Beller A, Mouvery A, Furlong HM, Maitre NL (2015) Neuroimaging identifies increased manganese deposition in infants receiving parenteral nutrition. Am J Clin Nutr 102(6):1482–1489. https://doi.org/10.3945/ajcn.115.116285
20. Erikson KM, Aschner M (2006) Increased manganese uptake by primary astrocyte cultures with altered iron status is mediated primarily by divalent metal transporter. Neurotoxicology 27(1):125–130. https://doi.org/10.1016/j.neuro.2005.07.003
21. Aschner M, Gannon M, Kimelberg HK (1992) Manganese uptake and efflux in cultured rat astrocytes. J Neurochem 58(2):730–735
22. Wedler FC, Denman RB (1984) Glutamine synthetase: the major Mn(II) enzyme in mammalian brain. Curr Top Cell Regul 24:153–169
23. Liao SL, Chen CJ (2001) Manganese stimulates stellation of cultured rat cortical astrocytes. NeuroReport 12(18):3877–3881
24. Weisiger RA, Fridovich I (1973) Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. J Biol Chem 248(13):4793–4796
25. Yan BC, Gong C, Song J, Krausz T, Tretiakova M, Hyjek E, Al-Ahmadie H, Alves V, Xiao SY, Anders RA, Hart JA (2010) Arginase-1: a new immunohistochemical marker of hepatocytes and hepatocellular neoplasms. Am J Surg Pathol 34(8):1147–1154. https://doi.org/10.1097/PAS.0b013e3181e5dffa
26. Gotoh T, Araki M, Mori M (1997) Chromosomal localization of the human arginase II gene and tissue distribution of its mRNA. Biochem Biophys Res Commun 233(2):487–491. https://doi.org/10.1006/bbrc.1997.6473
27. Yu H, Iyer RK, Kern RM, Rodriguez WI, Grody WW, Cederbaum SD (2001) Expression of arginase isozymes in mouse brain. J Neurosci Res 66(3):406–422. https://doi.org/10.1002/jnr.1233
28. Bichell TJV, Wegrzynowicz M, Tipps KG, Bradley EM, Uhouse MA, Bryan M, Horning K, Fisher N, Dudek K, Halbesma T, Umashanker P, Stubbs AD, Holt HK, Kwakye GF, Tidball AM, Colbran RJ, Aschner M, Neely MD, Di Pardo A, Maglione V, Osmand A, Bowman AB (1863) Reduced bioavailable manganese causes striatal urea cycle pathology in Huntington's disease mouse model. Biochim Biophys Acta Mol Basis Dis 6:1596–1604. https://doi.org/10.1016/j.bbadis.2017.02.013
29. Bowman AB, Aschner M (2014) Considerations on manganese (Mn) treatments for in vitro studies. Neurotoxicology 41:141–142. https://doi.org/10.1016/j.neuro.2014.01.010
30. Tjalkens RB, Zoran MJ, Mohl B, Barhoumi R (2006) Manganese suppresses ATP-dependent intercellular calcium waves in astrocyte networks through alteration of mitochondrial and endoplasmic reticulum calcium dynamics. Brain Res 1113(1):210–219. https://doi.org/10.1016/j.brainres.2006.07.053
31. Pfalzer AC, Bowman AB (2017) Relationships between essential manganese biology and manganese toxicity in neurological disease. Curr Environ Health Rep 4(2):223–228. https://doi.org/10.1007/s40572-017-0136-1
32. Quadri M, Federico A, Zhao T, Breedveld GJ, Battisti C, Delnooz C, Severijnen LA, Di Toro Mammarella L, Mignarri A, Monti L, Sanna A, Lu P, Punzo F, Cossu G, Willemsen R, Rasi F, Oostra BA, van de Warrenburg BP, Bonifati V (2012) Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease. Am J Hum Genet 90(3):467–477. https://doi.org/10.1016/j.ajhg.2012.01.017
33. Leyva-Illades D, Chen P, Zogzas CE, Hutchens S, Mercado JM, Swaim CD, Morrisett RA, Bowman AB, Aschner M, Mukhopadhyay S (2014) SLC30A10 is a cell surface-localized manganese efflux transporter, and parkinsonism-causing mutations block its intracellular trafficking and efflux activity. J Neurosci 34(42):14079–14095. https://doi.org/10.1523/Jneurosci.2329-14.2014
34. Belanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab 14(6):724–738. https://doi.org/10.1016/j.cmet.2011.08.016
35. Sidoryk-Wegrzynowicz M, Aschner M (2013) Manganese toxicity in the central nervous system: the glutamine/glutamate-gamma-aminobutyric acid cycle. J Intern Med 273(5):466–477. https://doi.org/10.1111/joim.12040
36. Bak LK, Schousboe A, Waagepetersen HS (2006) The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. J Neurochem 98(3):641–653. https://doi.org/10.1111/j.1471-4159.2006.03913.x
37. Sidoryk-Wegrzynowicz M, Lee E, Aschner M (2012) Mechanism of Mn(II)-mediated dysregulation of glutamine-glutamate cycle: focus on glutamate turnover. J Neurochem 122(4):856–867. https://doi.org/10.1111/j.1471-4159.2012.07835.x

38. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A (1999) Synthesis of vesicular GABA from glutamine involves TCA cycle metabolism in neocortical neurons. J Neurosci Res 57(3):342–349
39. Laake JH, Slyngstad TA, Haug FM, Ottersen OP (1995) Glutamine from glial cells is essential for the maintenance of the nerve terminal pool of glutamate: immunogold evidence from hippocampal slice cultures. J Neurochem 65(2):871–881
40. Karki P, Lee E, Aschner M (2013) Manganese neurotoxicity: a focus on glutamate transporters. Ann Occup Environ Med 25(1):4. https://doi.org/10.1186/2052-4374-25-4
41. Takumi Y, Matsubara A, Danbolt NC, Laake JH, Storm-Mathisen J, Usami S, Shinkawa H, Ottersen OP (1997) Discrete cellular and subcellular localization of glutamine synthetase and the glutamate transporter GLAST in the rat vestibular end organ. Neuroscience 79(4):1137–1144
42. Broer S (2014) The SLC38 family of sodium-amino acid co-transporters. Pflugers Arch 466(1):155–172. https://doi.org/10.1007/s00424-013-1393-y
43. Boulland JL, Osen KK, Levy LM, Danbolt NC, Edwards RH, Storm-Mathisen J, Chaudhry FA (2002) Cell-specific expression of the glutamine transporter SN1 suggests differences in dependence on the glutamine cycle. Eur J Neurosci 15(10):1615–1631
44. Broer A, Brookes N, Ganapathy V, Dimmer KS, Wagner CA, Lang F, Broer S (1999) The astroglial ASCT2 amino acid transporter as a mediator of glutamine efflux. J Neurochem 73(5):2184–2194
45. Deitmer JW, Broer A, Broer S (2003) Glutamine efflux from astrocytes is mediated by multiple pathways. J Neurochem 87(1):127–135. https://doi.org/10.1046/j.1471-4159.2003.01981.x
46. Chaudhry FA, Schmitz D, Reimer RJ, Larsson P, Gray AT, Nicoll R, Kavanaugh M, Edwards RH (2002) Glutamine uptake by neurons: interaction of protons with system a transporters. J Neurosci 22(1):62–72. https://doi.org/10.1523/Jneurosci.22-01-00062.2002
47. Albrecht J, Sidoryk-Wegrzynowicz M, Zielinska M, Aschner M (2010) Roles of glutamine in neurotransmission. Neuron Glia Biol 6(4):263–276. https://doi.org/10.1017/S1740925x11000093
48. Dachet F, Liu J, Ravits J, Song F (2019) Predicting disease specific spinal motor neurons and glia in sporadic ALS. Neurobiol Dis. https://doi.org/10.1016/j.nbd.2019.104523
49. Yuan S, Zhang ZW, Li ZL (2017) Cell death-autophagy loop and glutamate-glutamine cycle in amyotrophic lateral sclerosis. Front Mol Neurosci 10:231. https://doi.org/10.3389/fnmol.2017.00231
50. Sako W, Abe T, Izumi Y, Harada M, Kaji R (2016) The ratio of N-acetyl aspartate to glutamate correlates with disease duration of amyotrophic lateral sclerosis. J Clin Neurosci 27:110–113. https://doi.org/10.1016/j.jocn.2015.08.044
51. Bonifacino T, Cattaneo L, Gallia E, Puliti A, Melone M, Provenzano F, Bossi S, Musante I, Usai C, Conti F, Bonanno G, Milanese M (2017) In-vivo effects of knocking-down metabotropic glutamate receptor 5 in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neuropharmacology 123:433–445. https://doi.org/10.1016/j.neuropharm.2017.06.020
52. Milanese M, Giribaldi F, Melone M, Bonifacino T, Musante I, Carminati E, Rossi PI, Vergani L, Voci A, Conti F, Puliti A, Bonanno G (2014) Knocking down metabotropic glutamate receptor 1 improves survival and disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neurobiol Dis 64:48–59. https://doi.org/10.1016/j.nbd.2013.11.006
53. Fang T, Al Khleifat A, Meurgey JH, Jones A, Leigh PN, Bensimon G, Al-Chalabi A (2018) Stage at which riluzole treatment prolongs survival in patients with amyotrophic lateral sclerosis: a retrospective analysis of data from a dose-ranging study. Lancet Neurol 17(5):416–422. https://doi.org/10.1016/S1474-4422(18)30054-1
54. Ghoddoussi F, Galloway MP, Jambekar A, Bame M, Needleman R, Brusilow WS (2010) Methionine sulfoximine, an inhibitor of glutamine synthetase, lowers brain glutamine and glutamate in a mouse model of ALS. J Neurol Sci 290(1–2):41–47. https://doi.org/10.1016/j.jns.2009.11.013
55. Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. Neuron 39(6):889–909. https://doi.org/10.1016/s0896-6273(03)00568-3
56. Hudnell HK (1999) Effects from environmental Mn exposures: a review of the evidence from non-occupational exposure studies. Neurotoxicology 20(2–3):379–397
57. Ambrosi G, Cerri S, Blandini F (2014) A further update on the role of excitotoxicity in the pathogenesis of Parkinson's disease. J Neural Transm (Vienna) 121(8):849–859. https://doi.org/10.1007/s00702-013-1149-z
58. Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur J Pharmacol 698(1–3):6–18. https://doi.org/10.1016/j.ejphar.2012.10.032
59. Koutsilieri E, Rethwilm A, Scheller C (2007) The therapeutic potential of siRNA in gene therapy of neurodegenerative disorders. J Neural Transm Suppl 72:43–49
60. Benedetto A, Au C, Aschner M (2009) Manganese-induced dopaminergic neurodegeneration: insights into mechanisms and genetics shared with Parkinson's disease. Chem Rev 109(10):4862–4884. https://doi.org/10.1021/cr800536y
61. Lu Y, Zhang X, Zhao L, Yang C, Pan L, Li C, Liu K, Bai G, Gao H, Yan Z (2018) metabolic disturbances in the striatum and substantia Nigra in the onset and progression of MPTP-induced parkinsonism model. Front Neurosci 12:90. https://doi.org/10.3389/fnins.2018.00090
62. Lu E, Sarkar S, Raymick J, Paule MG, Gu Q (2018) Decreased Mcl-1 protein level in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice. Brain Res 1678:432–439. https://doi.org/10.1016/j.brainres.2017.11.014
63. Hazell AS, Itzhak Y, Liu H, Norenberg MD (1997) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) decreases glutamate uptake in cultured astrocytes. J Neurochem 68(5):2216–2219. https://doi.org/10.1046/j.1471-4159.1997.68052216.x
64. Walton HS, Dodd PR (2007) Glutamate-glutamine cycling in Alzheimer's disease. Neurochem Int 50(7–8):1052–1066. https://doi.org/10.1016/j.neuint.2006.10.007
65. Madeira C, Vargas-Lopes C, Brandao CO, Reis T, Laks J, Panizzutti R, Ferreira ST (2018) Elevated glutamate and glutamine levels in the cerebrospinal fluid of patients with probable Alzheimer's disease and depression. Front Psychiatry 9:561. https://doi.org/10.3389/fpsyt.2018.00561
66. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR (1991) Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 88(23):10540–10543. https://doi.org/10.1073/pnas.88.23.10540
67. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM et al (1995) Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem 65(5):2146–2156. https://doi.org/10.1046/j.1471-4159.1995.65052146.x
68. Le Prince G, Delaere P, Fages C, Lefrancois T, Touret M, Salanon M, Tardy M (1995) Glutamine synthetase (GS) expression is reduced in senile dementia of the Alzheimer type. Neurochem Res 20(7):859–862

69. Starke-Reed PE, Oliver CN (1989) Protein oxidation and proteolysis during aging and oxidative stress. Arch Biochem Biophys 275(2):559–567. https://doi.org/10.1016/0003-9861(89)90402-5
70. Kulijewicz-Nawrot M, Sykova E, Chvatal A, Verkhratsky A, Rodriguez JJ (2013) Astrocytes and glutamate homoeostasis in Alzheimer's disease: a decrease in glutamine synthetase, but not in glutamate transporter-1, in the prefrontal cortex. ASN Neuro 5(4):273–282. https://doi.org/10.1042/AN20130017
71. Greenamyre JT, Penney JB, Young AB, D'Amato CJ, Hicks SP, Shoulson I (1985) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. Science 227(4693):1496–1499. https://doi.org/10.1126/science.2858129
72. Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L (1996) Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann Neurol 40(5):759–766. https://doi.org/10.1002/ana.410400512
73. Li S, Mallory M, Alford M, Tanaka S, Masliah E (1997) Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. J Neuropathol Exp Neurol 56(8):901–911. https://doi.org/10.1097/00005072-199708000-00008
74. Takahashi K, Kong Q, Lin Y, Stouffer N, Schulte DA, Lai L, Liu Q, Chang LC, Dominguez S, Xing X, Cuny GD, Hodgetts KJ, Glicksman MA, Lin CL (2015) Restored glial glutamate transporter EAAT2 function as a potential therapeutic approach for Alzheimer's disease. J Exp Med 212(3):319–332. https://doi.org/10.1084/jem.20140413
75. Mutkus L, Aschner JL, Fitsanakis V, Aschner M (2005) The in vitro uptake of glutamate in GLAST and GLT-1 transfected mutant CHO-K1 cells is inhibited by manganese. Biol Trace Elem Res 107(3):221–230. https://doi.org/10.1385/Bter:107:3:221
76. Erikson KM, Dorman DC, Lash LH, Aschner M (2008) Duration of airbome-manganese exposure in rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. Neurotoxicology 29(3):377–385. https://doi.org/10.1016/j.neuro.2007.12.007
77. Eid A, Mhatre I, Richardson JR (2019) Gene-environment interactions in Alzheimer's disease: a potential path to precision medicine. Pharmacol Ther 199:173–187. https://doi.org/10.1016/j.pharmthera.2019.03.005
78. Meyer-Baron M, Schaper M, Knapp G, Lucchini R, Zoni S, Bast-Pettersen R, Ellingsen DG, Thomassen Y, He S, Yuan H, Niu Q, Wang XL, Yang YJ, Iregren A, Sjogren B, Blond M, Laursen P, Netterstrom B, Mergler D, Bowler R, van Thriel C (2013) The neurobehavioral impact of manganese: results and challenges obtained by a meta-analysis of individual participant data. Neurotoxicology 36:1–9. https://doi.org/10.1016/j.neuro.2013.02.003
79. Bouchard MF, Sauve S, Barbeau B, Legrand M, Brodeur ME, Bouffard T, Limoges E, Bellinger DC, Mergler D (2011) Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect 119(1):138–143. https://doi.org/10.1289/ehp.1002321
80. Ye Q, Kim J (2015) Loss of hfe function reverses impaired recognition memory caused by olfactory manganese exposure in mice. Toxicol Res 31(1):17–23. https://doi.org/10.5487/TR.2015.31.1.017
81. Deng Y, Xu Z, Xu B, Xu D, Tian Y, Feng W (2012) The protective effects of riluzole on manganese-induced disruption of glutamate transporters and glutamine synthetase in the cultured astrocytes. Biol Trace Elem Res 148(2):242–249. https://doi.org/10.1007/s12011-012-9365-1
82. Lee E, Sidoryk-Wegrzynowicz M, Farina M, Rocha JB, Aschner M (2013) Estrogen attenuates manganese-induced glutamate transporter impairment in rat primary astrocytes. Neurotox Res 23(2):124–130. https://doi.org/10.1007/s12640-012-9347-2
83. Johnson J Jr, Pajarillo EAB, Taka E, Reams R, Son DS, Aschner M, Lee E (2018) Valproate and sodium butyrate attenuate manganese-decreased locomotor activity and astrocytic glutamate transporters expression in mice. Neurotoxicology 64:230–239. https://doi.org/10.1016/j.neuro.2017.06.007
84. Pajarillo E, Johnson J Jr, Kim J, Karki P, Son DS, Aschner M, Lee E (2018) 17beta-estradiol and tamoxifen protect mice from manganese-induced dopaminergic neurotoxicity. Neurotoxicology 65:280–288. https://doi.org/10.1016/j.neuro.2017.11.008
85. Sidoryk-Wegrzynowicz M, Lee ES, Ni MW, Aschner M (2010) Manganese-Induced downregulation of astroglial glutamine transporter SNAT3 involves ubiquitin-mediated proteolytic system. Glia 58(16):1905–1912. https://doi.org/10.1002/glia.21060
86. Susarla BTS, Robinson MB (2008) Internalization and degradation of the glutamate transporter GLT-1 in response to phorbol ester. Neurochem Int 52(4–5):709–722. https://doi.org/10.1016/j.neuint.2007.08.020
87. Karki P, Webb A, Smith K, Johnson J, Lee K, Son DS, Aschner M, Lee E (2014) Yin Yang 1 is a repressor of glutamate transporter EAAT2, and it mediates manganese-induced decrease of EAAT2 expression in astrocytes. Mol Cell Biol 34(7):1280–1289. https://doi.org/10.1128/Mcb.01176-13
88. Karki P, Kim C, Smith K, Son DS, Aschner M, Lee E (2015) Transcriptional regulation of the astrocytic excitatory amino acid transporter 1 (EAAT1) via NF-kappa B and Yin Yang 1 (YY1). J Biol Chem 290(39):23725–23737. https://doi.org/10.1074/jbc.M115.649327
89. Milatovic D, Yin ZB, Gupta RC, Sidoryk M, Albrecht J, Aschner JL, Aschner M (2007) Manganese induces oxidative impairment in cultured rat astrocytes. Toxicol Sci 98(1):198–205. https://doi.org/10.1093/toxsci/kfm095
90. Sidoryk-Wegrzynowicz M, Lee E, Albrecht J, Aschner M (2009) Manganese disrupts astrocyte glutamine transporter expression and function. J Neurochem 110(3):822–830. https://doi.org/10.1111/j.1471-4159.2009.06172.x
91. Jentsch S, Schlenker S (1995) Selective protein-degradation—a journeys end within the proteasome. Cell 82(6):881–884. https://doi.org/10.1016/0092-8674(95)90021-7
92. Sidoryk-Wegrzynowicz M, Lee E, Ni MW, Aschner M (2011) Disruption of astrocytic glutamine turnover by manganese is mediated by the protein kinase C pathway. Glia 59(11):1732–1743. https://doi.org/10.1002/glia.21219
93. Balkrishna S, Broer A, Kingsland A, Broer S (2010) Rapid downregulation of the rat glutamine transporter SNAT3 by a caveolin-dependent trafficking mechanism in Xenopus laevis oocytes. Am J Physiol-Cell Ph 299(5):C1047–C1057. https://doi.org/10.1152/ajpcell.00209.2010
94. Pawlik TM, Souba WW, Sweeney TJ, Bode BP (2000) Phorbol esters rapidly attenuate glutamine uptake and growth in human colon carcinoma cells. J Surg Res 90(2):149–155. https://doi.org/10.1006/jsre.2000.5872
95. Sofroniew MV (2014) Astrogliosis. Cold Spring Harb Perspect Biol 7(2):a020420. https://doi.org/10.1101/cshperspect.a020420
96. Barhoumi R, Faske J, Liu X, Tjalkens RB (2004) Manganese potentiates lipopolysaccharide-induced expression of NOS2 in C6 glioma cells through mitochondrial-dependent activation of nuclear factor kappaB. Brain Res Mol Brain Res 122(2):167–179. https://doi.org/10.1016/j.molbrainres.2003.12.009
97. Liu X, Buffington JA, Tjalkens RB (2005) NF-kappaB-dependent production of nitric oxide by astrocytes mediates apoptosis in differentiated PC12 neurons following exposure to manganese and cytokines. Brain Res Mol Brain Res 141(1):39–47. https://doi.org/10.1016/j.molbrainres.2005.07.017
98. Liu XH, Sullivan KA, Madl JE, Legare M, Tjalkens RB (2006) Manganese-induced neurotoxicity: the role of astroglial-derived

nitric oxide in striatal interneuron degeneration. Toxicol Sci 91(2):521–531. https://doi.org/10.1093/toxsci/kfj150
99. Popichak KA, Afzali MF, Kirkley KS, Tjalkens RB (2018) Glial-neuronal signaling mechanisms underlying the neuroinflammatory effects of manganese. J Neuroinflamm 15(1):324. https://doi.org/10.1186/s12974-018-1349-4
100. Tjalkens RB, Popichak KA, Kirkley KA (2017) Inflammatory activation of microglia and astrocytes in manganese neurotoxicity. Adv Neurobiol 18:159–181. https://doi.org/10.1007/978-3-319-60189-2_8
101. Booth HDE, Hirst WD, Wade-Martins R (2017) The role of astrocyte dysfunction in Parkinson's disease pathogenesis. Trends Neurosci 40(6):358–370. https://doi.org/10.1016/j.tins.2017.04.001
102. Reed X, Bandres-Ciga S, Blauwendraat C, Cookson MR (2019) The role of monogenic genes in idiopathic Parkinson's disease. Neurobiol Dis 124:230–239. https://doi.org/10.1016/j.nbd.2018.11.012
103. Martin S, van Veen S, Holemans T, Demirsoy S, van den Haute C, Baekelandt V, Agostinis P, Eggermont J, Vangheluwe P (2016) Protection against mitochondrial and metal toxicity depends on functional lipid binding sites in ATP13A2. Parkinsons Dis 2016:9531917. https://doi.org/10.1155/2016/9531917
104. Qiao C, Yin N, Gu HY, Zhu JL, Ding JH, Lu M, Hu G (2016) Atp13a2 deficiency aggravates astrocyte-mediated neuroinflammation via NLRP3 inflammasome activation. CNS Neurosci Ther 22(6):451–460. https://doi.org/10.1111/cns.12514
105. Fleming SM, Santiago NA, Mullin EJ, Pamphile S, Karkare S, Lemkuhl A, Ekhator OR, Linn SC, Holden JG, Aga DS, Roth JA, Liou B, Sun Y, Shull GE, Schultheis PJ (2018) The effect of manganese exposure in Atp13a2-deficient mice. Neurotoxicology 64:256–266. https://doi.org/10.1016/j.neuro.2017.06.005
106. Sillence DJ (2007) New insights into glycosphingolipid functions—storage, lipid rafts, and translocators. Int Rev Cytol 262:151–189. https://doi.org/10.1016/S0074-7696(07)62003-8
107. Bandopadhyay R, Kingsbury AE, Cookson MR, Reid AR, Evans IM, Hope AD, Pittman AM, Lashley T, Canet-Aviles R, Miller DW, McLendon C, Strand C, Leonard AJ, Abou-Sleiman PM, Healy DG, Ariga H, Wood NW, de Silva R, Revesz T, Hardy JA, Lees AJ (2004) The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson's disease. Brain 127(Pt 2):420–430. https://doi.org/10.1093/brain/awh054
108. Lee E, Yin Z, Sidoryk-Wegrzynowicz M, Jiang H, Aschner M (2012) 15-Deoxy-Delta 12,14-prostaglandin J(2) modulates manganese-induced activation of the NF-kappaB, Nrf2, and PI3K pathways in astrocytes. Free Radic Biol Med 52(6):1067–1074. https://doi.org/10.1016/j.freeradbiomed.2011.12.016
109. Chen P, DeWitt MR, Bornhorst J, Soares FA, Mukhopadhyay S, Bowman AB, Aschner M (2015) Age- and manganese-dependent modulation of dopaminergic phenotypes in a *C. elegans* DJ-1 genetic model of Parkinson's disease. Metallomics 7(2):289–298. https://doi.org/10.1039/c4mt00292j
110. Harrington AJ, Hamamichi S, Caldwell GA, Caldwell KA (2010) *C. elegans* as a model organism to investigate molecular pathways involved with Parkinson's disease. Dev Dynam 239(5):1282–1295. https://doi.org/10.1002/dvdy.22231
111. Alexander AG, Marfil V, Li C (2014) Use of *Caenorhabditis elegans* as a model to study Alzheimer's disease and other neurodegenerative diseases. Front Genet. https://doi.org/10.3389/fgene.2014.00279
112. Kirienko NV, Mani K, Fay DS (2010) Cancer models in *Caenorhabditis elegans*. Dev Dyn 239(5):1413–1448. https://doi.org/10.1002/dvdy.22247
113. Dwyer DS (2018) Crossing the worm-brain barrier by using *Caenorhabditis elegans* to explore fundamentals of human psychiatric illness. Mol Neuropsychiatry 3(3):170–179. https://doi.org/10.1159/000485423
114. Katz M, Corson F, Iwanir S, Biron D, Shaham S (2018) Glia modulate a neuronal circuit for locomotion suppression during sleep in *C. elegans*. Cell Rep 22(10):2575–2583. https://doi.org/10.1016/j.celrep.2018.02.036
115. Rapti G, Li C, Shan A, Lu Y, Shaham S (2017) Glia initiate brain assembly through noncanonical Chimaerin-Furin axon guidance in *C. elegans*. Nat Neurosci 20(10):1350. https://doi.org/10.1038/nn.4630
116. Zogzas CE, Mukhopadhyay S (2017) Inherited disorders of manganese metabolism. Adv Neurobiol 18:35–49. https://doi.org/10.1007/978-3-319-60189-2_3