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Astrocytes in Aceruloplasminemia

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Abstract

In neurons, iron plays an important role in the signal transduction related to synaptic plasticity. The neuronal iron supply is tightly controlled and depends not only on transferrin-bound iron but also on non-transferrin-bound iron (NTBI). Ceruloplasmin is bound to the cell membranes of astrocytes, where it plays a role in iron efflux from astrocytes due to the activity of ferroxidase, which oxidizes ferrous iron after its transfer to the cell surface via ferroportin, and which delivers ferric iron to extracellular transferrin, which is transported to neurons. Aceruloplasminemia is an autosomal recessive neurodegenerative disorder in which iron accumulates in the brain due to the complete lack of ceruloplasmin ferroxidase activity. Redox-active iron accumulation was found to be more prominent in astrocytes than in neurons. Neurons take up iron from alternative sources of NTBI because astrocytes without ceruloplasmin cannot transport iron to transferrin. Neuronal cell loss may result from iron starvation in the early stage of aceruloplasminemia and may result from iron-mediated oxidation in the late stage of the condition. The excess iron in astrocytes can result in oxidative damage to these cells, thereby disrupting the neuronal cell protection offered by astrocytes in patients with aceruloplasminemia.

Keywords: ceruloplasmin, iron, ferroxidase, non-transferrin-bound iron (NTBI), neurodegeneration

1. The role of iron in brain

Iron is a bioactive metal that is essential for a normal brain function. It participates in a variety of cellular functions, including the biosynthesis of many neurotransmitters, myelin formation and electron transport, which sustains the brain's energy metabolism. On the other hand, excessive iron in the brain causes neuronal injury, because redox-active ferrous iron (Fe^{2+}) enhances oxidative stress due to the generation of the highly cytotoxic hydroxyl radical [1, 2]. A deficiency or excess of iron can cause impaired cellular functions and eventually cell death.

However, the precise mechanisms underlying the metabolism of iron and its regulation in the brain remain unknown. Iron deficiency in the developing human has been clearly established as a causative factor of long-term developmental and cognitive impairment. Late fetal and early postnatal iron deficiency is a condition that causes learning and memory impairments in humans, which persist after iron repletion [3–6]. Two other factors that are important in determining the degree of cognitive deficit are the magnitude and the duration of iron deficiency [7]. In contrast, while several prominent neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease, have been reported to be associated with the excessive accumulation of iron in specific brain regions, the relationship of this accumulation to the pathogenesis of these diseases is far from clear. The iron control in the brain is virtually independent of the rest of the body. Indeed, it has been reported that neither systemic iron-overload nor systemic iron deficiency has a significant effect on the brain in adulthood [1, 8].

Despite the critical and diverse role of iron in the brain function, the molecular and cellular details of neuronal iron metabolism remain poorly understood; however, current studies have started to uncover the participation of iron in signal transduction mechanisms related to synaptic plasticity and that iron is needed for long-term potentiation (LTP), and have provided a potential model to account for the learning and memory deficits exhibited by humans with iron deficiency. Iron-generated reactive oxygen species (ROS) could be a new class of molecules that act as second messengers in the signaling cascades related to synaptic plasticity, the putative cellular substrate of memory [9]. These ROS are involved in the calcium signaling initiated by the stimulation of NMDA receptors. On the other hand, an excess of iron, with the ensuing uncontrolled production of ROS, is detrimental to neuronal survival. In the presence of elevated iron, increased synaptic activity can cause iron overload and contribute to the development of cytotoxic effects [10], as was suggested to occur in Alzheimer's disease [11]. A direct coupling between synaptic activity and iron entry can properly address these requirements. This is a double-edged sword in that iron entry—if not properly controlled—represents a harmful condition, because Fe^{2+} is capable of catalyzing the Fenton reaction, which occurs primarily at the mitochondrial level with the production of the highly toxic hydroxyl radical [12]. Astrocytes that can uptake iron, and which thereby buffer its concentration in the synaptic environment, may play a protective role. Astrocytes are endowed with a strong detoxifying defense system that makes them more resistant to oxidative insults than neurons [13].

2. Ceruloplasmin

Ceruloplasmin consists of a single chain of 1046 amino acids and is a member of the multicopper oxidase family. This protein is a glycoprotein of the α_2 globulin fraction of the serum, and contains 95% of the copper in the plasma [14]. There were precisely six copper ions present in the molecule, and that there was an important three-copper catalytic center—the so-called 'trinuclear cluster'—which plays an important role in the oxidase reaction [15]. Despite the need for copper for the functions of ceruloplasmin, this protein plays no essential role in the transport or metabolism of copper. Copper depletion resulted in a marked decrease in the

circulating serum ceruloplasmin in association with iron deficiency anemia that could only be corrected by the administration of copper and by the accumulation of iron in parenchymal tissues, while the administration of exogenous ceruloplasmin resulted in the prompt release of iron from tissue with subsequent incorporation into circulating transferrin. The essential function of ceruloplasmin is as a ferroxidase, which utilizes the electron chemistry of bound copper ions to couple the oxidation of ferrous iron (Fe^{2+}) to the reduction of oxygen bound to the trinuclear cluster [16, 17].

Two isoforms of this protein are generated by alternative splicing: a secretory form (serum ceruloplasmin) and a glycosylphosphatidylinositol (GPI)-linked form [18]. The secretory form is mostly expressed in hepatocytes, while the GPI-linked form is expressed in the brain, liver, and several other organs [19]. Although the GPI-linked form is strongly expressed within the brain, several other tissues are known to express it at relatively low levels (i.e., the spleen, kidney, heart, and liver). In the brain, most ceruloplasmin is derived from astrocytes and is located on the surface of astrocytes in the GPI-linked form [20].

Serum transferrin-bound iron is endocytosed by brain endothelial cells, which are dependent on transferrin receptor 1, and iron is released to the brain interstitial fluid through the basolateral iron exporter, ferroportin. Extracellular iron is oxidized due to GPI-linked ceruloplasmin, which is found in the foot processes of astrocytes, and then iron binds to the transferrin and is transported to neurons [21]. The GPI-linked ceruloplasmin likely plays an important role in the mobilization of iron and the antioxidant effects in the central nervous system [20, 22]. GPI-linked ceruloplasmin may be associated with iron homeostasis and the antioxidant defense by protecting the central nervous system from iron-mediated free radical injury. The ferroxidase activity of GPI-linked ceruloplasmin is also essential for the stability of cell surface ferroportin [23, 24]. The importance of ceruloplasmin in human biology is underscored by aceruloplasminemia, which is an inherited disease of iron homeostasis. This disease reveals an essential role of ceruloplasmin in brain iron homeostasis. It is known that (1) ceruloplasmin regulates the efficiency of iron efflux, (2) ceruloplasmin functions as a ferroxidase and regulates the oxidation of ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), (3) ceruloplasmin does not bind to transferrin directly, (4) ceruloplasmin stabilizes the cell surface iron transporter, ferroportin, and (5) GPI-linked ceruloplasmin is the predominant form expressed in the brain [19].

3. Aceruloplasminemia

In 1987, we described the first case of aceruloplasminemia in a 52-year-old Japanese woman suffering from blepharospasm, retinal degeneration, and diabetes mellitus (DM) [25]. Subsequent evaluations revealed the presence of anemia and low serum iron concentrations, despite the fact that the patient had high levels of serum ferritin and marked iron accumulation in the brain and liver on T2-weighted magnetic resonance imaging (MRI, **Figure 1**), as well as the complete absence of serum ceruloplasmin. The lack of serum ceruloplasmin was

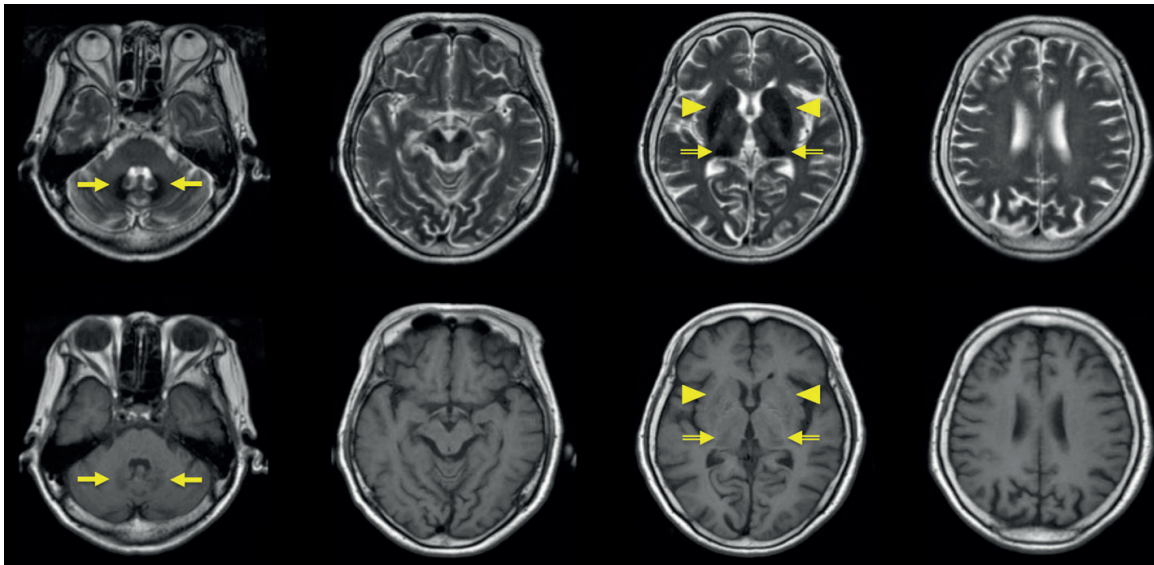


Figure 1. The brain MRI findings in a patient with aceruloplasminemia. T1-weighted (lower row) and T2-weighted (upper row) axial images of the brain showed signal attenuation of the dentate nucleus of the cerebellum (arrows), basal ganglia (arrowheads), and thalami (double arrows).

inherited in an autosomal recessive fashion. A direct connection between iron accumulation in both the brain and liver and the complete absence of serum ceruloplasmin was hypothesized. A genetic analysis of the ceruloplasmin gene revealed that this patient was homozygous, with a 5-base insertion in exon 7 (c.1286_90insTACAC), which resulted in a frame shift mutation and a truncated open reading frame [26]. The clinical findings and the identification of a mutation in the ceruloplasmin gene confirmed that the disorder was a novel disorder of iron metabolism, which resulted from a lack of ceruloplasmin in the serum. The disorder was termed aceruloplasminemia (MIM 604290). The patient died from pancreatic cancer at 66 years of age. We examined the pathological studies that were performed in this case [27]. Brown pigmentation of the basal ganglia was observed in a coronal section of the brain (**Figure 2**). Severe iron deposition was observed in the basal ganglia, thalamus, and cerebellum, and neuronal loss was observed in the regions with the highest iron accumulation. The iron deposition in astrocytes was more severe than that in neurons (**Figure 2**). The globular structures (inclusions) were identified in the cerebral cortices as well as the basal ganglia. These structures included many oxidatively damaged proteins that were derived from astrocytes. Intense redox-active iron deposition was mainly demonstrated in the inclusions in the astrocytes (**Figure 2**).

Aceruloplasminemia is classified as an inherited neurodegenerative disorder with systemic iron-overload syndrome. The clinical manifestations of aceruloplasminemia are the triad of retinal degeneration, DM, and neurological signs/symptoms [27]. The neurological manifestations (in order of frequency) include ataxia, involuntary movement, cognitive dysfunction and parkinsonism; these correspond to the specific regions of brain iron accumulation. These symptoms generally appear in the fourth or fifth decade of life. More than 40% of involuntary movement is dystonia, and approximately 25% of cases exhibit chorea and choreoathetosis.

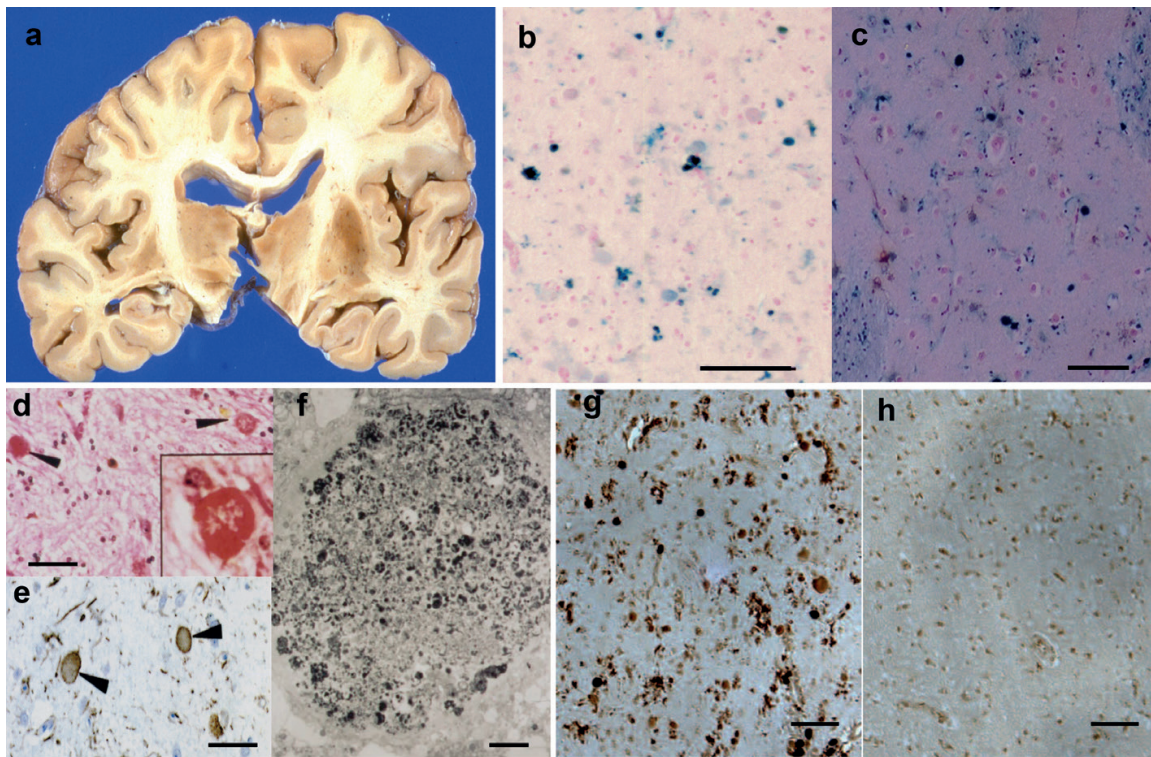


Figure 2. The histopathological findings in a patient with aceruloplasminemia. A coronal section of the brain shows brown pigmentation of the basal ganglia (a). Severe ferric iron deposition is noted in the putamen (b: Berlin blue stain). Iron deposition is mainly observed in the astrocytes, and a small amount of ferric iron is seen in the neurons (c: Berlin blue staining + H&E staining). Globular structures, indicated by arrowheads, are seen in the astrocytes (d: H&E staining) and were positive for anti-HNE antibody (e). The electron microscopic findings of the globular structures indicate that they contain many electron-dense bodies (f). The marked accumulation of redox-active iron is shown in the globus pallidus of an aceruloplasminemic brain (g) in comparison to the brain of a control subject (h) (redox-active iron staining: A modified Perl's technique). Scale bars: b-e, g, h = 100 μ m; f = 5 μ m.

The cognitive dysfunction includes forgetfulness, mental slowing, and apathy. The neuro-imaging studies of aceruloplasminemia patients are strongly supported by the characteristic abnormally low intensity on MRI, reflecting the accumulation of iron in the liver and brain. In Japan, the prevalence of aceruloplasminemia was estimated to be approximately one per 2,000,000 in individuals with non-consanguineous parents [28]. Genetic testing can confirm the diagnosis. Worldwide, genetic analyses of patients with aceruloplasminemia have identified more than 50 distinct mutations in the ceruloplasmin gene [29]. The majority of mutations are truncated mutations, which lead to the formation of a premature stop codon. The human ceruloplasmin gene contains 20 exons. The ferroxidase activity of ceruloplasmin is dependent upon the trinuclear copper cluster, the ligands for which are encoded by exon 18 [30]. The truncated mutations identified are predicted to result in the formation of a protein lacking the copper cluster sites that are presumed to be critical for the enzymatic function. The clinical phenotype in most patients shows little variation, regardless of the specific mutation [31]. The precise pathogenesis of iron deposition in the brain has been unclear, but evidence from several studies suggests that the enhanced oxidative stress induced by excess iron causes neuronal cell death [32, 33].

4. The role of astrocytes in aceruloplasminemia

In the brain of aceruloplasminemia patients, abnormal astrocytes were more frequently observed in the basal ganglia, where the accumulation of iron was marked than that in the frontal cortex. Intense ferrous iron deposition was demonstrated in the inclusions, many of which were positive for glia fibrillary acidic protein (GFAP) and which were stained by anti-4-hydroxynoneal (HNE) antibody. GFAP is most severely modified by oxidative stress in the brains of patients with aceruloplasminemia [34]. Intense ferrous iron deposition was demonstrated in the inclusions. The morphological changes in the astrocytes may be related to iron-induced tissue damage.

GPI-linked ceruloplasmin is bound to the cell membranes of astrocytes, where it plays an important role in the mobilization of iron from the blood to the extracellular space in the brain through astrocytes due to the ferroxidase activity. Ferroportin is a cell membrane-bound protein that is expressed in the brain as well as several organs and which transports intracellular ferrous iron to transferrin via the oxidization of ferrous iron to ferric iron via the ferroxidase of ceruloplasmin. GPI-linked ceruloplasmin likely plays an important role in the mobilization of iron from astrocytes to neurons. A ceruloplasmin homolog, hephaestin, is also expressed on neurons and functions as a ferroxidase to interact with neuronal ferroportin. Ceruloplasmin and ceruloplasmin/hephaestin knockout mice exhibited a neurodegenerative phenotype and retinal degeneration, consistent with that seen in aceruloplasminemia patients [35, 36]. The brain requires iron at concentrations that are several times higher than that obtained from the blood in order to maintain its normal function [2]. Taken together, the known functions of iron metabolic molecules suggest the presence of a cycle of iron storage and reutilization within the brain [37]. The neuronal iron supply is tightly controlled and mainly neurons take up iron from transferrin and alternative sources of non-transferrin-bound iron (NTBI). The pathological findings in the brain of patients with aceruloplasminemia included severe iron deposition in both the astrocytes and neurons, and neuronal loss. The iron accumulation observed in the neurons indicates that the neurons take up significant amounts of iron due to alternative sources of NTBI, because astrocytes without any expression of ceruloplasmin cannot transport iron to the transferrin that binds to transferrin receptor 1 on neurons. A ceruloplasmin-deficient model showed that neuronal cell loss may result from iron starvation in regions where the iron in astrocytes is not effectively mobilized for the uptake into neurons, and the accumulation of excess iron in astrocytes may also result in oxidative damage to these cells, with the subsequent loss of the glial-derived growth factors that are critical for neurons [36]. Neuronal cell injury may therefore result from iron deficiency in the early stage as well as iron-mediated oxidation in the late stage (**Figure 3**). NTBI is mainly composed of Fe^{3+} that is loosely bound to buffering molecules (mainly citrate and ascorbate). The high concentrations of ascorbate in the cerebrospinal fluid, which are up to 100 times higher than the concentrations in plasma, result in a reducing environment that increases the ratio $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio in NTBI [38]. Thus, in addition to its physiological role in the brain, NTBI can acquire a pathological relevance, as NTBI levels increase during aging. NTBI has been thought to be an important contributor to the pathogenesis of cancer, cardiovascular diseases, and neurodegenerative disorders. The high propensity of NTBI to induce the generation of ROS makes it a potentially toxic form of iron; it is responsible for cellular damage not only at the plasma membrane level but also toward different intracellular organelles [39]. High levels of NTBI

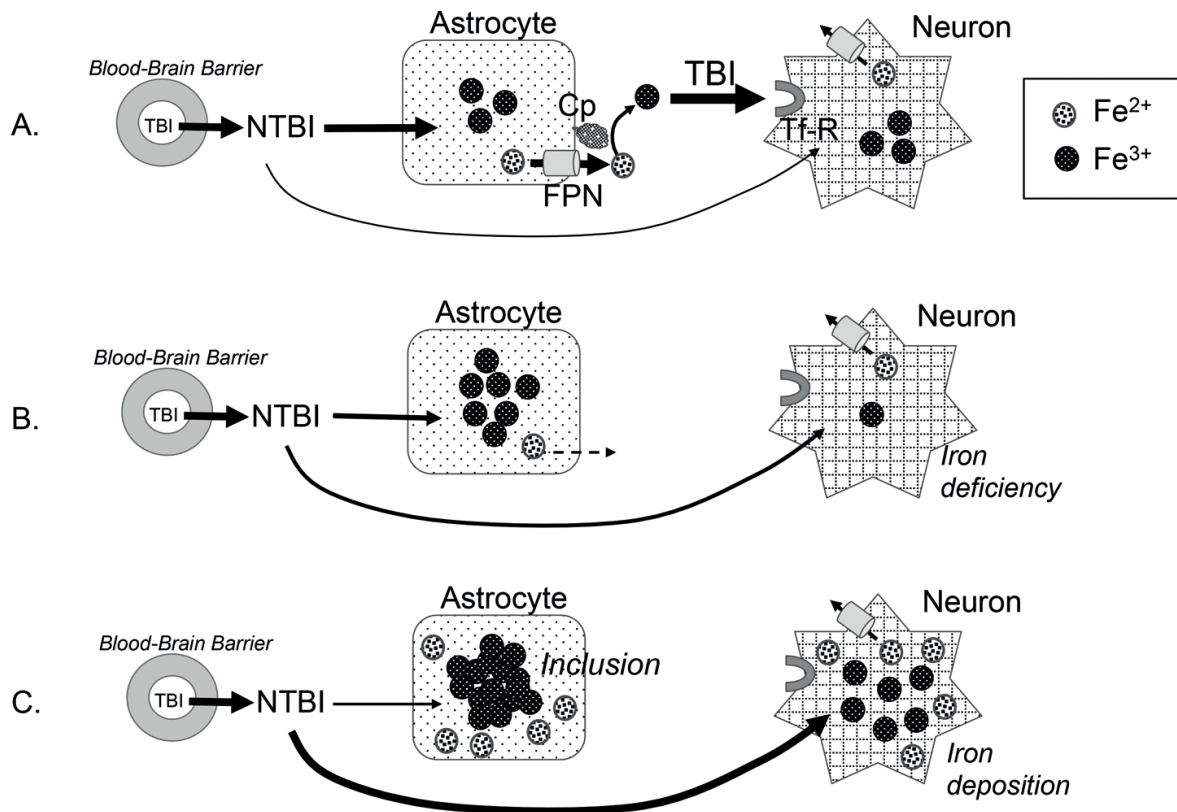


Figure 3. A model of the iron metabolic cycle in the brain. In the normal brain (a), iron may be recycled between astrocytes and neurons. Transferrin acts as a shuttle to deliver iron from astrocytes to neurons. The GPI-linked ceruloplasmin on astrocytes is a ferroxidase that mediates the oxidation of ferrous iron and its subsequent transfer to transferrin. Neurons take up the transferrin-bound iron and also take up iron from alternative sources (non-transferrin-bound iron; NTBI). Hephaestin also plays a role as a ferroxidase and interacts with neuronal ferroportin. In the brain of a patient with aceruloplasminemia, neuronal cell loss may result from iron deficiency in regions where the iron in astrocytes cannot be mobilized for the uptake into neurons in the early stage of the disease (b). Iron accumulation is subsequently observed in neurons as well as astrocytes, since neurons take up iron from NTBI, not from transferrin, in the late stage of the disease (c), because astrocytes without GPI-linked ceruloplasmin cannot transport iron to transferrin. TBI, transferrin-bound iron; NTBI, non-transferrin-bound iron; FPN, iron transporter of ferroportin; Cp, ceruloplasmin; Tf-R, transferrin receptor 1.

accumulate in the brains of patients with neurodegenerative disorders, including patients with Parkinson's disease and Alzheimer's disease [40]. Oxidative stress, which is closely related to the increased iron levels in the brain, and which may also occur due to defects in the antioxidant defense mechanism, is widely believed to be associated with neuronal cell death in patients with these diseases [41]. Astrocyte dysfunction may contribute to neuronal cell loss, in addition to the direct effects of free radicals on neurons. GPI-linked ceruloplasmin may be associated with astrocyte homeostasis and neuronal survival in the brain.

5. Conclusion

Astrocytes play an important role in iron homeostasis in the brain. They participate in the synaptic plasticity through the supply of iron to neurons and by buffering iron. The excess iron in astrocytes can result in oxidative damage to these cells, thereby disrupting the neuronal cell protection offered by astrocytes in patients with aceruloplasminemia.

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