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Overview of the gut-liver-brain axis with particular emphasis on ferroptosis

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Abstract. Ferroptosis is a form of cellular death involved in the origin, progression, but also regulation of several human diseases. Its regulatory role in the gut-liver-brain axis (GLBA) has not been clarified. Therefore, we sought to summarize the possible correlations between ferroptosis and the GLBA. In this review, we first introduce the phenotype and the main mechanisms of this relatively newly described form of regulated cell death. Then, we analyse the anatomy of the GLBA, describing the connections between the gut and the liver, followed by the anatomical pathways from the gut to the brain and from the liver to the brain. After the morphological aspects, we summarize the main biological modulators of the GLBA, highlighting their physiological and pathological roles. In the end, we discuss in detail the regulatory role of ferroptosis on neuroinflammation and oxidative stress along GLBA, highlighting the key aspects that could be of significant clinical importance as future diagnostic and therapeutic targets.

Keywords: ferroptosis, gut-brain axis, neuroinflammation.

1. FERROPTOSIS: A NEW FORM OF NON-APOPTOTIC CELL DEATH

Ferroptosis is an iron-dependent, lipid peroxidation-driven form of regulated cell death (RCD). First discovered by Brent R. Stockwell et al. (2012), it was described as erastin-induced cell death characterized by a peculiar phenotype.[1] Morphological (such as loss of membrane integrity, swollen of the cytoplasm and smaller mitochondria), biochemical and genetic features distinguish ferroptosis from the other types of RCD described to date[2]. The

regulation of this unique process of cell death is closely linked to the interplay between iron, lipid and amino acid metabolism resulting in oxidative perturbations of the intracellular microenvironment and loss of redox homeostasis. Recent advances in the discovery of ferroptosis markers revealed how ferroptosis is the main feature of a series of physiological processes, including tumor suppression pathways and adequate immunological response.[3] The relevance of ferroptosis is further highlighted by its role in the development of pathological conditions, spanning from kidney injuries and endocrine imbalances to neurodegenerative disorders[4], including Alzheimer's Disease (AD) and Parkinson's Disease (PD) along with synucleinopathy.[5]

1.1. Molecular and morphological phenotypes

The buildup of peroxidized lipids, resulting in the unrepaired damage of cell membranes, is the ultimate driver of ferroptosis.[6,7] The contribution of different organelles in the biochemical evolution of ferroptosis explains the main morphological changes affecting these subcellular compartments. Mitochondria can add up to cysteine-starvation mediated GSH-depletion-induced damage by means of electrons leakage from the cellular respiration machinery, which would facilitate the initiation of a Fenton reaction by producing hydrogen peroxide. The endoplasmic reticulum, being the critical site of lipid peroxidation, is fundamental for clustering of oxidized Poly-Unsaturated Fatty Acids (PUFA) and phospholipids (PLs) leading to progression of ferroptosis.[7] Overall, the modifications at the cellular and subcellular levels include: mitochondria shrinkage and cristae reduction or disappearance, increased endoplasmic reticulum viscosity and ER membrane stiffness, loss of plasma membrane structural and functional integrity, and possible formation of pores that can promote membrane rupture.[8–11]

1.2. Mechanism

The molecular mechanism of ferroptosis is driven by the increase of intracellular ferrous iron (Fe^{2+}), capable of subsequently promoting both non-enzymatic and enzymatic lipid peroxidation by initiating a Fenton reaction or by exploiting its role as a critical cofactor of lipoxygenases.[12] The detrimental effects of lipid peroxidation fall into two, possibly overlapping, categories:[13] direct dampening of cellular membranes stability and formation of lipid pores, with consequent increase the permeability of the membranes, or the activation of downstream signaling pathways that result in membrane perforation.[14,15]

Iron homeostasis, primarily controlled by the hepatic secretion of hepcidin,[16] is met by a tightly balanced exchange of iron between extracellular and intracellular compartments, in which the transition metal is found as ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron, respectively.[17] The expansion of the cellular labile iron pool (LIP), reaching more than 5% of total cell iron,[18] promotes reactive oxygen species (ROS) formation through the reaction of ferrous iron with hydrogen peroxide, according to Fenton chemistry.[19] The rise in intracellular Fe^{2+} could be secondary to a variety of conditions, which include: (i) an increase of transferrin receptor (TFR1) expression, (ii) RAS mutations or NCOA4-mediated ferritinophagy, which induces a decrease in ferritin levels,[20,21] and (iii) excessive degradation of heme by heme oxygenase 1 (HO-1)[22] (Figure 1). Lipid peroxidation can occur through both non-enzymatic and enzymatic pathways.

Non-enzymatic lipid peroxidation

Non-enzymatic lipid peroxidation consists of free radical driven reactions. Initiation requires a Fenton reaction to take place: the abundant ferrous iron moieties react with hydrogen peroxide derived from oxidoreductase activity, such as that of cytochromes P450 oxidoreductase (POR) and P450 reductase (CPR) localized on the smooth endoplasmic reticulum, producing ROS. [23] The hydroxyl radicals then extract hydrogen from the bis-allylic position of poly-unsaturated fatty acids (PUFAs) to form lipid radicals, which further react with molecular oxygen (O_2) to produce lipid peroxy-radicals. More hydrogen moieties are removed from PUFAs to form lipid hydroperoxides and new lipid peroxy-radicals, the latter providing a way of amplification of the oxidation reaction[24,25] (Figure 2).

Enzymatic peroxidation

Enzymatic lipid peroxidation, as the name implies, consists of a series of enzymatically driven reactions. In the endoplasmic-reticulum-associated subcellular compartments, selective oxidation of phosphatidylethanolamines (PEs) followed by lipids oxygenation takes place.[26] Firstly acyl-CoA synthetases, such as ACSL4 (especially acting on arachidonic acid), activate free fatty acids (FFAs) by adding to them a coenzyme A moiety. Then, lysophospholipid acyltransferases, such as LPCAT3, catalyze the transfer of the fatty acyl chain from fatty acyl-CoA and incorporate into PEs to form various classes of phospholipids. Finally, lipoxygenases familiy (LOXs) generate doubly and triply oxygenated (15-hydroperoxy)-diacylated PE compounds[26,27] (Figure 3).

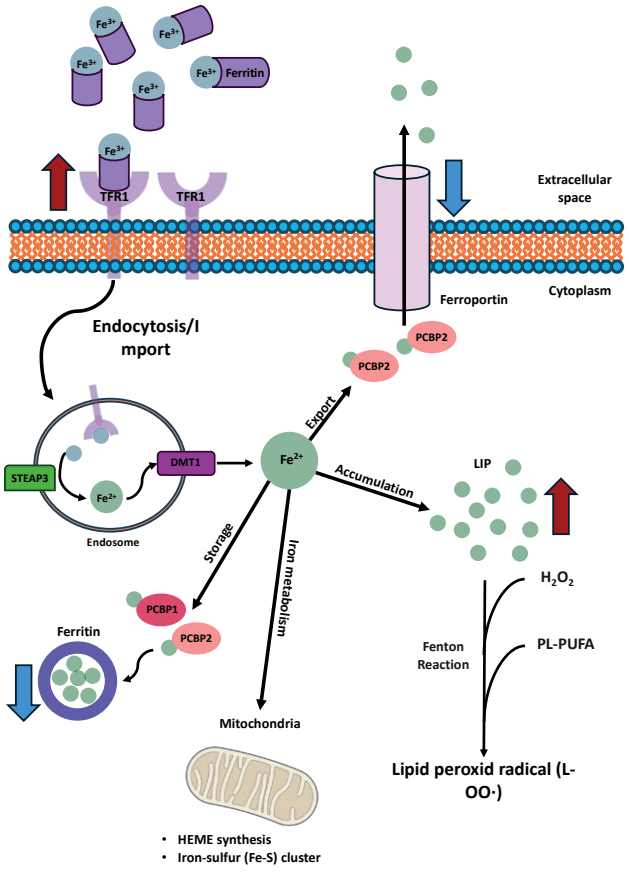

Figure 1. The regulatory mechanisms of ferroptosis. The extracellular domain of transferrin receptors (TFR1) binds Trasferrin-Fe3+ on the cell surface, triggering the receptor-mediated endocytosis. In the endosome, STEAP3 reduced the Fe3+ into Fe2+, which is transported in the cytosol by the DMT1. Elevated levels of ferrous ions (Fe2+) were found in the cytoplasm due to: (i) reduction of the ferroportin activity, (ii) increase accumulation of Fe2+ as cellular Labile Iron Poll (LIP) in the cytosol, and (iii) increase of iron-mediated mitochondrial homeostasis. At last, reduced expression of ferritin due to ferritinophagy could increase Fe2+ in the cytoplasm. Imbalance between iron uptake, storage and export may increase the susceptibility of cells to ferroptosis.

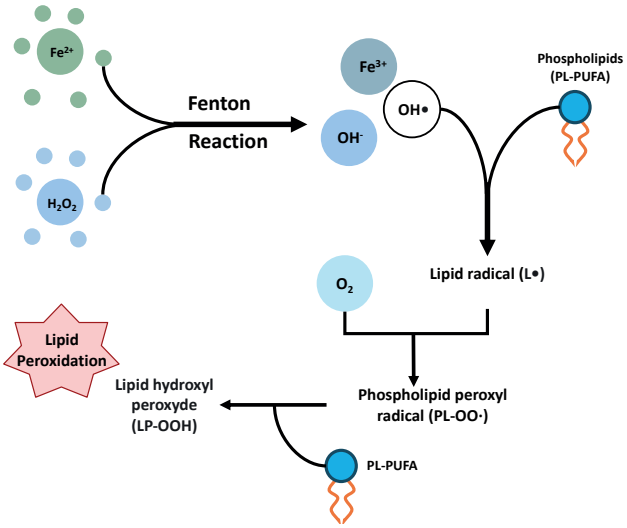

Figure 2. Non-enzymatic lipid peroxidation (autoxidation) requires a Fenton reaction, but the precise role of iron in the process of lipid peroxidation is still being debated whether this peroxidation occurs in an enzymatic or non-enzymatic way.

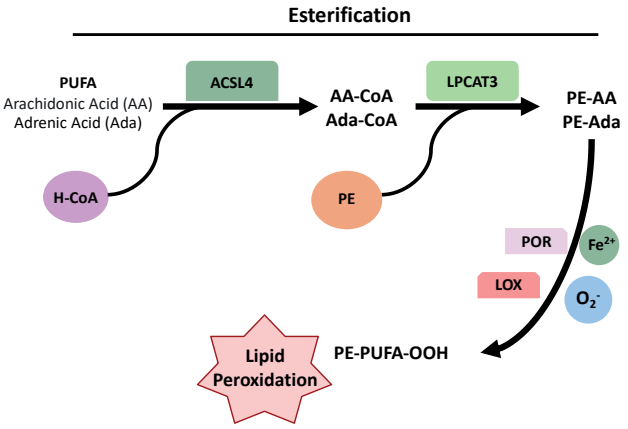

Figure 3. Enzymatic lipid peroxidation requires numerous enzymatically reactions. Although the effectors leading to ferroptosis in physiological conditions are not well known, but lipid peroxidation and GPX4 enzymology are critical for this process. When the homeostatic control of the steady state between LOOH formation and reduction is lost, lipid peroxidation is activated and ferroptosis is executed.

2. THE ANATOMY OF THE GUT-LIVER-BRAIN AXIS

2.1. The gut-liver axis

The gut-liver axis (GLA) concept was first introduced by Marshall in 1998 and refers to the connection between the gastrointestinal (GI) tract and the liver. This axis is characterized by a complex network of bidirectional anatomical and functional interactions between the GI tract and the liver. Over the years, the GLA has gained increasing attention due to its implications in various pathologies. Key components of this axis include portal circulation, gut microbiota, intestinal tight junctions, bile acids, and mucosal hormones.[28–30]

Portal circulation

Most of the venous blood from the GI tract, primarily from the small and large intestines, drains into the portal vein. This vein transports nutrient-rich blood, along with signalling molecules produced by the gut

microbiota, neuroendocrine and immune cells of the gut, directly to the liver. Moreover, the portal circulation carries to the liver the products of hemocatheresis occurring in the spleen, including globins, heme and iron produced by the catabolism of haemoglobin.

Gut microbiota

The gut hosts diverse microbial communities that play essential roles in maintaining intestinal integrity and producing signalling molecules. A balanced microbiota is critical to produce various metabolites, particularly short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate.[31] Acetate, absorbed in the proximal colon, is rapidly transported to the liver, where it contributes to cholesterol biosynthesis.[32] Propionate, a substrate for lipogenesis, gluconeogenesis and protein synthesis in the liver,[33] was observed to inhibit hepatic cholesterol synthesis.[34] Butyrate was demonstrated to be central for the health of colon enterocytes by providing energy to colonic epithelial cells.[35] However, recent studies suggest that excessive accumulation of butyrate induces cholestasis, hepatocyte death, and neutrophil-driven inflammation in the liver, potentially leading to icteric hepatocellular carcinoma.[36] Additionally, SCFAs, especially butyrate, stimulate the release of fasting-induced adipocyte factor (FIAF) from L cells in the gut;[37] FIAF subsequently inhibits lipoprotein lipase (LPL) activity, preventing triglyceride accumulation in both adipose tissue and liver. [7,38] Therefore, the microbiota plays a central role in the GLA and dysbiosis can lead to an imbalance of signalling molecules, contributing to liver damage. Not surprisingly, an imbalanced gut microbiota can result in increased intestinal ethanol production, contributing to the development of non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD), today included in metabolic dysfunction-associated steatotic liver disease (MASLD).[39,40]

Tight Junctions (TJs)

Tight junctions (TJs) are components of the gut mucosal barrier, a multifaceted system of physical, chemical, microbial, and immunological defences that limit the spread of intestinal antigens.[41] TJs are composed of proteins such as claudins, TJ-associated marvel proteins (TAMPs), junctional adhesion molecules (JAMs), and zona occludens-1 (ZO-1).[42–44] In the context of the GLA, the loss of barrier integrity, for example due to inflammation, can increase portal leakage of lipopolysaccharide (LPS), with the activation of a well-characterized pathogen-associated molecular pattern (PAMPs). LPS activates Kupffer cells, the liver's resident macrophages, are highly sensitive to LPS, that activate it.[45] LPS-activated Kupffer cells promote liver inflammation and fibrosis by driving the transcription of pro-inflammatory cytokines and the activation of the stellate cells into the Disse's space.[31,46] Under healthy conditions, the portal vein carries small amounts of pathogens and bacteria, which are effectively managed by the liver's immune system. However, pathological states can result in an excessive influx of microorganisms into the portal vein, contributing to liver disease.[47,48]

In neurodegenerative conditions, α-syn aggregates can travel via portal circulation from the gut to the liver. Moreover, aggregated α-syn can be either a consequence as a driver of bowel inflammation with alteration of the intestinal barrier and increased passage of LPS to the liver.[49,50]

Bile

Bile is a digestive fluid secreted by the hepatocytes and stored in the gallbladder to reach the duodenum. Within the context of the GLA, bile acids play a crucial signalling role. The gut microbiota converts primary bile acids, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), into secondary bile acids, including lithocholic acid (LCA) and deoxycholic acid (DCA).[51] Both primary and secondary bile acids are absorbed by enterocytes and enter the portal circulation. Bile acids act as signalling molecules by interacting with nuclear farnesoid X receptors (FXR) in both intestine and liver, regulating cholesterol, lipid, and energy metabolism.[52] Reabsorbed bile acids can also activate Takeda G protein-coupled receptor 5 (TGR5 a membrane receptor for bile acid), causing multiple effects in hepatic stellate cells, Kupffer cells, cholangiocytes, and enterocytes.[53,54] Activation and transdifferentiation of hepatic stellate cells into myofibroblasts can promote liver fibrosis.[55] Another important receptor, Shingosine-1-phosphate receptor 2 (S1PR2), is present in the liver and bile ducts and is activated by conjugated bile acids such as taurine or glycine-conjugated bile acids[53] Inhibition of S1PR2 has shown promising results as a potential treatment for cholestatic liver diseases. Knockout mice for S1PR2 showed reduced inflammation and hepatic fibrosis in liver.[56] Dysregulation of the gut-liver circulation is evident in cirrhotic patients, where depletion of bacterial populations responsible for bile acid dehydroxylation has been observed.[57] Additionally, gut microbiota can convert choline, a bile component, into trimethylamine N-oxide (TMAO), a toxic metabolite associated with dysbiosis and hepatic steatosis.[53,58] Lastly, bile contains IgA, bicarbonate, and antibacterial molecules, which have bacteriostatic properties and contribute to the regulation of gut microbiota composition.[47]

Mucosal hormones

The gut mucosa contains cells capable of releasing hormones, such as substance P, vasoactive intestinal peptide (VIP) and serotonin as well as fibroblast growth factor 15 (FGF15), which regulates bile-acids synthesis, hepatic glucose, and lipid metabolism.[59] FGF15 (which is equivalent in humans to FGF19)[60] also plays a crucial role in regulating postprandial glucose and energy metabolism by modulating gluconeogenesis.[61] The glucagon-like peptide 1 (GLP-1) is an incretin hormone secreted by L cells of ileum and colon. GLP-1 is involved in the regulation of glucose metabolism in the liver, stomach emptying and feeding.[62] Secretin, another crucial hormone, secreted by S cells in the duodenum, has been shown: (i) to regulate biliary secretion and proliferation[63]and, (ii) to modulate the brown adipose tissue (BAT)-brain metabolic crosstalk.[64] Moreover, one of the endocrine mechanisms responsible for weight regain includes the brain-gut axis, which supports food intake via the production of several gastrointestinal hormones, such as ghrelin, leptin and cholecystokinin (CKK).[65]

2.2. The gut-brain axis

The concept of the gut-brain axis (GBA) was first proposed by Michael D. Gershon in the late 20th century. The idea suggested a bidirectional interaction between the GI tract and brain. The importance of this axis is now emerging. Changes in GI biochemical and physiological pathways have been observed in a growing range of CNS disorders, such as PD, in which GI dysfunction often precedes of decades the onset of neurological symptoms.[66] Generally, the gut-brain axis is mediated by the enteric nervous system (ENS), autonomic nervous system (ANS), hypothalamus-pituitary-adrenal (HPA) axis, the enteroendocrine systems, and the immune system.[53]

ENS anatomy

The GI tract is innervated by number of neurons (estimated at 200-600 million) that exceeds the total number of neurons in the spinal cord. This neuronal system, specific of the gut, is classified as the ENS, also known as the meta sympathetic nervous system. Studies suggested that these neurons are evolutionarily older than those of the central nervous system, and the ENS is capable of functioning independently through various reflexes without input from the CNS and the ANS. Consequently, the ENS is often referred to as the body's "second brain".[67–69] Anatomically, the ENS can be considered a complex network of neuronal connections and ganglia embedded in the layers of the gut wall. These groups of neurons can be subdivided into two plexuses: the Auerbach's (myenteric) plexus, located between the circular and longitudinal layers of the muscularis externa, and the Meissner's (submucosal) plexus, situated within the submucosa.[70] The ENS comprises a complex network of neurons, some of which have been classified, such as: intrinsic primary afferent neurons, excitatory and inhibitory motor neurons, ascending and descending interneurons, secretomotor/vasodilator neurons, and intestinofugal neurons. [71,72]

ENS - ANS cross talk

Although the ENS can function independently, it is still strongly influenced by the ANS (both sympathetic and parasympathetic). The sympathetic system reaches mostly the myenteric plexus, whereas the post ganglionic neurons of the parasympathetic nervous system (mostly constituted by hepatic and celiac branches of the vagus nerve) are found both within the myenteric and submucosal plexuses (Figure 4). The importance of the ANS and ENS in the context of the gut can be understood by considering the numerous functions in which they are involved: gut motility, gut permeability, epithelial fluid homeostasis, luminal osmolarity, bile secretion, carbohydrate concentration, mechanical distortion of the mucosa, bicarbonate production, mucus production and secretion, as well as mucosal immune responses and handling of intestinal fluids.[73,74]

The neuroactive components of the GBA

A diverse array of neuroactive substances exists within the GBA, encompassing gut-derived hormones, neuroactive molecules, metabolites produced by gut microbiota, and various microbial products.[75] Notable examples include bacteria-derived metabolites such as short-chain-fatty-acids (SCFAs), gamma-aminobutyric acid (GABA), serotonin (5-HT), glutamate, acetylcholine, dopamine, norepinephrine, and gut neuropeptides, including peptide YY (PYY), GLP-1, CCK, and ghrelin.[53] A central role in detecting these substances is played by vagal terminal afferent fibres, which have been described in three locations: in the terminals ending of the intestinal muscular layers, in the GI mucosa, and in a subset of enteroendocrine cells (now referred as neuropods) that form synapses with vagal neurons.[76] Due to their wide range of receptor expression, vagal afferents are considered polymodal, meaning that they can detect multiple types of stimuli, including mechanical, chemical, and hormonal signals.[77] Signals from these afferent nerves ascend towards the nucleus tractus solitarius (NTS) in the CNS[78] where they are relayed to other brainstem nuclei and forebrain structures.[79] Nervous communica-

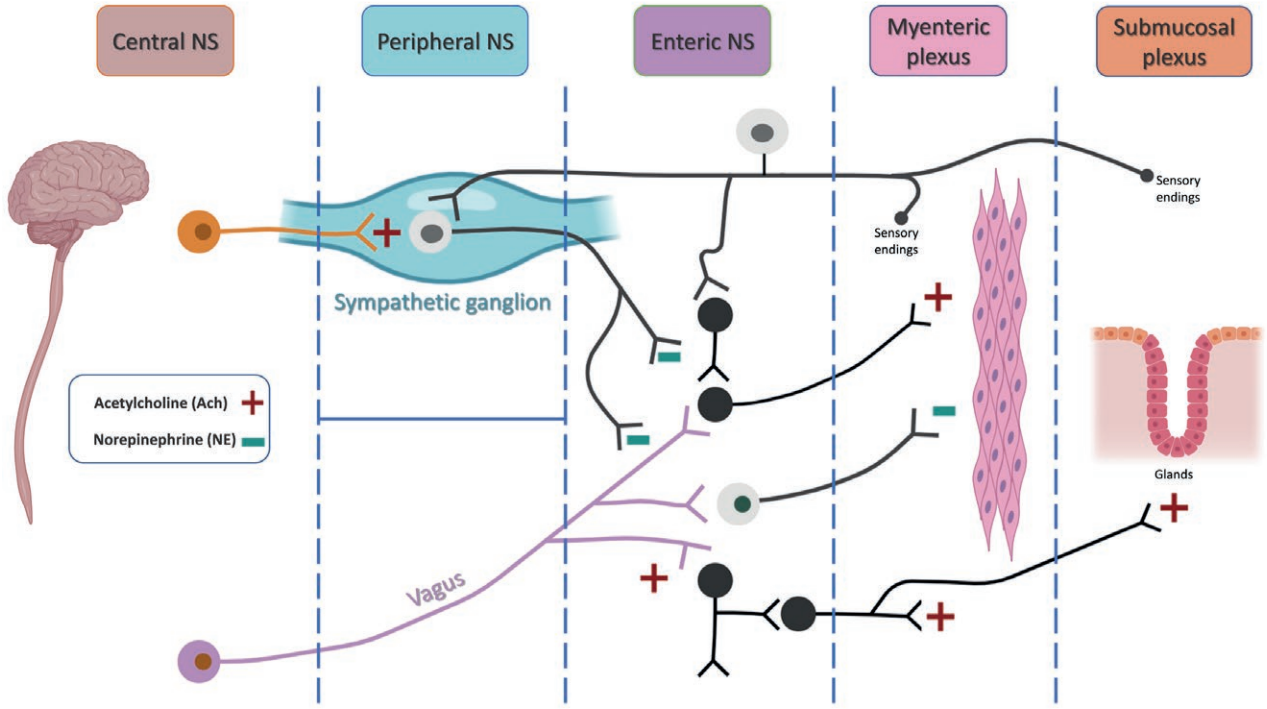

Figure 4. Representative scheme of the anatomical nervous stations. Organization of the myenteric and submucosal plexuses in which are evident the sites of synaptic excitation (+) and inhibition (-). Scheme modified by Kleman JA 1995. Barr's The Human Nervous System: An Anatomical Viewpoint.

tion is bilateral, thus substances targeting the CNS are also released by the ENS and ANS, including GABA, glutamate, acetylcholine, dopamine, norepinephrine, and other bioactive amines.[80]

The microbiota in the gut brain axis

As previously mentioned, the role of gut microbiota is becoming increasingly central in biomedical research, evolving the concept of the GBA into the microbiota-gut-brain axis.[81,82] In addition to interacting with neuronal terminals in the gut, microbial metabolites can enter the circulation and directly affect the CNS. Therefore, the composition of the gut microbiota and its related products, are crucial for the interactions between gut and brain, as well as for the determination of different neurological and psychiatric disorders. Several examples emphasize the molecular pathways involved in this interaction: SCFAs can cross the blood-brain-barrier (BBB) and reduce LPS-induced neurological inflammation in primary microglia and the hippocampus, as well as decrease circulating pro-inflammatory cytokines.[83–85] When imbalances in gut microbiota occur, like in patients affected by irritable bowel syndrome (IBS), the production of secondary bile acids decreases, leading to increased inflammation in the brain.[53] Lastly, it has been observed that trimethylamine N-oxide (TMAO, a metabolite of the gut microbiota) accelerates brain aging and causes age-associated cognitive impairments. [86]

The HPA system in the gut brain axis

The HPA axis is one of the body's primary systems for regulating the release of cortisol and other stress hormones. This response is also mediated by several components, including gut microbiota, the vagus nerve, and the immune system. Studies on germ-free (GF) mice have demonstrated that these mice exhibit an exaggerated HPA response when exposed to stress, releasing more adrenocorticotropic hormone (ACTH) and cortisol.[87,88] In humans, patients with IBS show higher levels of ACTH and cortisol in response to stress, along with altered microbiota composition.[89–91] The HPA axis interacts with other gut-brain communication routes, including the vagus nerve. Studies in rodents indicate that stimulating the vagus nerve increases corticotropin-releasing factor (CRF) production in the brain and raises ACTH and corticosterone levels.[92] The HPA axis also connects to the immune system, where stress and inflammation can influence gut-brain signalling. Stress increases gut permeability, allowing bacteria to cross

into the body, triggering an immune response that activates in turn the HPA axis.[93]

Enteroendocrine system

Enteroendocrine cells (EECs), though comprising only 1% of all gut epithelial cells, are vital for maintaining gut balance due to the various functions of the molecules they release.[94] There are multiple types of EECs, all of which act as sensors that help in regulating the processes such as insulin secretion and food intake based on gut contents.[94] Two well-studied types of EECs in the context of the gut-brain axis are enteroendocrine L cells and enterochromaffin cells.

L cells release hormones like GLP-1 and PYY, which reduce hunger and regulate food intake.[95] These hormones act on receptors in both gut and brain, either directly or indirectly through the vagus nerve, to signal feelings of satiety also involving the circuits of the parabrachial nucleus.[96,97] L cells also communicate with the ENS through neuropods, allowing for fast and precise signalling between the gut and brain.[97–99] The activation of L cells in different parts of the gut depends on what is contained in the lumen: in the upper gut, they are triggered by nutrients like carbohydrates and fats, while in the lower gut they are driven by bacterial metabolites like SCFAs, which can stimulate GLP-1 and PYY secretion.[100–102]

Enterochromaffin cells produce most of the serotonin (5-HT) out of the CNS, which is important for intestinal motility, pain perception, and inflammatory response.[103] Although serotonin from the gut doesn't directly affect the brain due to the BBB, it may influence gut-brain communication through vagal signals[103] and inflammation [104–106]

2.3. The liver-brain axis

Increasing attention is being given to the liver's connection with the brain, which occurs primarily through two main routes: neuronal connections and vascular pathways.[107] Understanding these complex neural circuits is essential to appreciate the liver's involvement in conditions that affect both the liver and brain, including neurodegenerative disorders.[108,109]

Liver brain nervous anatomical connection

The liver, a vital organ for metabolism, detoxification, and nutrient storage, is regulated by the ANS, comprising the sympathetic and parasympathetic branches. Autonomic nerve fibers enter the liver through the hilum, forming plexuses around the liver's primary blood vessels – an anterior plexus encircling the hepatic artery and a posterior plexus surrounding the portal vein.[108]

Sympathetic fibers to the liver originate from preganglionic neurons in the thoracic spinal cord (T7–T12), traveling through the splanchnic nerves and synapsing in the celiac and superior mesenteric ganglia. From there, postganglionic sympathetic fibers innervate various structures, including the hepatic artery, portal vein, and bile ducts.[110,111] Parasympathetic innervation occurs via the hepatic branches of the left vagus nerve, which also innervates the bile ducts, portal vein, duodenum, and portions of the pancreas.[112–114]

In animal species, parasympathetic innervation is generally limited to the portal triad, while sympathetic innervation varies. In humans they reach the hepatocytes.[115]

The sympathetic nervous system predominantly regulates catabolic processes, while the parasympathetic system supports anabolic functions.[116] For example, vagal nerve pathways modulate hepatic lipid metabolism, influencing very low-density lipoprotein (VLDL) triglyceride secretion and reducing lipid accumulation in the liver.[117]

Additionally, to adrenaline, noradrenaline and Ach, hepatic neurons synthesize and release various neurotransmitters and neuropeptides, including neuropeptide Y, substance P, VIP, glucagon-like peptide, somatostatin, neurotensin, and serotonin, all of which further influence liver and systemic homeostasis.[118,119]

Hepatic afferent nerves, particularly from the vagus nerve, sense the liver's microenvironment and relay signals to the NTS, where feedback is sent to the liver via parasympathetic and sympathetic nerves.[120,121] These signals are also transmitted to higher brain regions, such as the hypothalamus and limbic system, influencing metabolic regulation and organ function.[122]

Liver brain vascular communication

In addition to neural circuits, liver and brain communicate through several vascular mechanisms, including BBB permeability, immune modulation, epigenetic alterations and amyloid β metabolism.

BBB permeability: Pro-inflammatory cytokines, including TNF-α and IL-1β, increase BBB permeability, allowing substances like ammonia, xenobiotics, and inflammatory cytokines to penetrate the brain, exacerbating neuroinflammation.[123] Excessive activation of microglia due to these cytokines attracts monocytes to the brain parenchyma, creating chronic inflammation in conditions as liver failure.[124,125] This is evident in chronic liver diseases, where neuroinflammatory processes contribute to neurological pathologies, such as hepatic encephalopathy, which occurs in approximately 30–45% of patients with cirrhosis.[126] Cholestatic mouse models,

such as BDL, show debilitating CNS symptoms defined as sickness behaviors. This behavior appears to be related to elevated levels of circulating and liver-synthesized IL-6. Regulatory T cells (T-regs) appear to be responsible for modulating IL-6 synthesis and inhibiting the activity of circulating monocytes.[127]

Epigenetic alterations: An example of how a metabolite produced by the liver can act centrally is β-hydroxybutyrate, a ketone body capable of crossing the BBB via specific monocarboxylate transporters and able to inhibit histone deacetylases (HDACs) the most important epigenetic control enzymes.[128] Elevated β-hydroxybutyrate levels in the brain increase brain-derived neurotrophic factor (BDNF), which plays a therapeutic role in neurodegenerative diseases and mental health disorders like depression.[129,130]

Amyloid β: The liver is central to the peripheral metabolism of Aβ, and dysregulation of Aβ clearance is a critical factor in the pathogenesis of AD. The connection between the liver and brain is thus closely linked to the progression of AD.[131,132]

α-Synuclein: Accumulation of α-synuclein in the liver may start liver inflammation and fibrosis[133] or represents a mechanism of clearance, which prevent a massive transmission of α-synuclein aggregates from the gut to the brain.[134,135] For this reason, liver could be directly involved in progression of PD either by cytokines release as by regulation of the transmission and clearance of α-synuclein aggregates.

2.4. The gut - liver - brain axis (GLBA)

The GLBA represents a network of bidirectional communication among the GI tract, the liver, and the brain, highlighting the interdependence of these organs in maintaining homeostasis and in contributing also to cognitive process. Understanding the GLBA is primary to elucidate the pathophysiology and the mechanisms of certain diseases, and to develop innovative therapeutic strategies that target this multifaceted network.

Studies suggested that different pathological conditions inducing dysbiosis could have acute or chronic systemic consequences, such as a weakened of the immune system, inflammation or central disorders, as well as neuropsychiatric disorders, including anxiety, depression or neurodegenerative conditions.[136,137] Furthermore, events, such as maternal stress to lack of breastfeeding, mode of delivery (vaginal VS cesarean) and antibiotic exposure, are major players in the development of acute infections and long-term dysbiosis that are associated to chronic pathological conditions, including asthma, diabetes, neurodevelopmental disorders and IBS.[138,139]

In addition, dysbiosis can also impact the function of the HPA, a fundamental part of the limbic system, involved in both emotional behavior and memory consolidation[140,141]. At last, the role of dysbiosis in both a cause and consequence of the "leaky gut", a condition associated to many autoimmune disorders where intestinal permeability is increased and pathogens as well as toxins can enter more freely into the human body, is well documented.[142]

3. PRINCIPAL BIOCHEMICAL MODULATORS ALONG THE GUT-BRAIN AXIS

3.1. Serotonin

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter.[143] Historically, its roles have been mostly associated with the regulation of basic functions of the CNS, including sleep, mood and body temperature. Its deficits are present in numerous mental disorders.[144] Despite this common knowledge, it has been demonstrated that less than 5% of the total body's serotonin is in the CNS,[145] while most of it is hosted by the intestine.[146] 5-HT is produced mostly by enterochromaffin cells in the intestinal lining, which regulates many functions of the GI tract, including motility and permeability, as well as local secretions and ENS development [147].

Alterations of serotonin levels have been found in several diseases, including IBS,[148] hepatic conditions[149] and psychiatric disorders,[150] underlying the importance of the GBA and how serotonin pathways affect it. Recent studies have shown that commensal bacteria participate in the production and modulation of intestinal serotonin.[151] Consequently, prebiotics and probiotics can alter both synthesis and release of serotonin, actively impacting both GI and nervous system.[152] Moreover, although enteric 5-HT can both act locally and enter the bloodstream, divergent data on the capacity of 5-HT to cross the BBB are available.[153,154] Despite this, 5-HT has been shown to alter the permeability of the BBB.[155] Study demonstrated that the endothelium of the choroid plexus, which in healthy conditions is permeable to large (70 kDa) circulating molecules, upon intestinal inflammation acts as a vascular barrier (PVB), by closing its accessibility to inflammatory and bacterial molecules.[156] Recent study hypothesizes the capacity of neurotransmitters produced in the gut to cross the choroid plexus.[157] Therefore, it can be said that alterations in serotonin pathways can be both the cause and the consequence of GI or CNS diseases, as GI tract and brain are deeply linked, regulate each other, and are in charge for the control of emotional and stress responses.

As previously mentioned, numerous therapeutic options for several diseases affect serotonin metabolism, from its secretion to its release or reuptake. Due to the high prevalence of serotonin in the GI tract, such therapies can significantly impact GI functions as the use of serotonin reuptake inhinitors.[158] People affected by IBS exhibit altered levels of post-prandial plasma serotonin, both in children [159] and adults,[160] and it has been hypothesized that such individuals may have a genetic predisposition for decreased serotonin reuptake transporter (SERT) expression.[160] Potential therapeutic options are being studied via serotonin-related signaling pathways and the gut-liver axis, in the regulation of obesity,[161] MASLD, and hepatic steatosis. [162] Studies involving serotonin agonists have been shown to benefit patients affected by IBS suffering from constipation, by increasing intestinal mobility and secretion.[103] Moreover, tricyclic antidepressants that were used in numerous psychiatric conditions play a role in both mood and GI symptoms in patients affected by depression as well as IBD[163]. Furthermore, alterations in serotonin signaling can exacerbate gastrointestinal symptoms in stressed individuals, emphasizing the connection between psychological mechanisms and GI function.[164] The serotonin produced by guts significantly influences the brain through immune function, vagal nerve stimulation, neuroendocrine feedback, and the HPA axis, but the relationship between serotonin levels in the brain and depression remains unclear.[165]

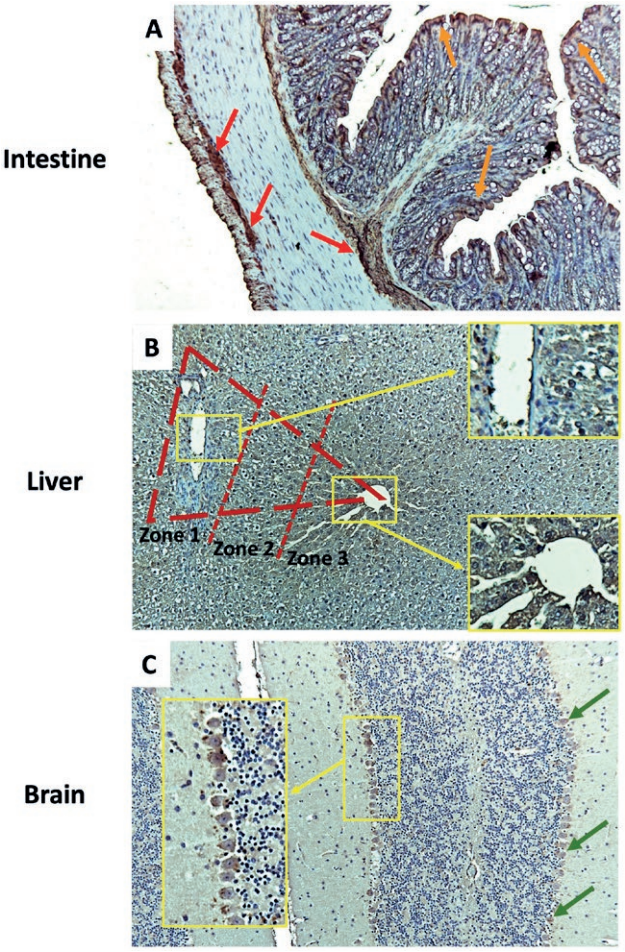

Figure 5. GLP-1 expression (Abcam AB22625) along the gut - liver - brain axis. GLP-1 is released from enteroendocrine cells of the lower intestine and exerts anorectic and antimotility actions.[167] In fact, GLP-1 expression at the level of colon is high in the intestinal epithelium (orange arrows) and in the myenteric and submucosal plexes (red arrows) **[A]**. In the hepatic parenchima exists a different gradient of expression inside the acinus, with an increase from the zone 1 towards zone 3, where it has an insulin-like activity, on glucagon-induced glycogenolysis[168] **[B]**. In the brain, dense immunoreactivity fills the cell bodies of the efferent neurons, such as the Purkinje cells in the cerebellum (green arrows) **[C]**. OM 10x

3.2. GLP-1 and GIP

GLP-1 and glucose dependent insulinotropic polypeptide (GIP) are incretin hormones secreted by their EECs.[166] These cells constitute a small portion of the total intestinal cell population (about 1%). Despite this, they represent the largest endocrine organ of the human body, and they are distributed along the entire GI tract (Figure 5). These are divided into three different subgroups, each specialized in the production of a different gut hormone: (i) L cells, which secrete (secreting GLP-1), (ii) I cell (secreting cholecystokinin [CCK]), and (iii) K cells, which secrete (secreting GIP).

Incretin GLP-1 is present at low levels in a fasting state, and it increases within the first few minutes after food ingestion, reaching a peak of about 15 *p*mol/l at around 60-90 minutes after a meal.[166] The GLP-1 incretin is characterized by a very short half-life, corresponding to about 1 to 2 minutes. Therefore, only about 12% of gut-derived GLP-1 enters the systemic circulation.[167] This could relate to the possibility that GLP-1 could activate vagal terminals innervating, the gut and the hepato-portal region, mediating also a signal from the brain to the pancreatic β-cells through vasovagal reflexes that induce insulin secretion.[168] In fact, the common hepatic, celiac and gastric branches have been shown to contribute to the glucoregulatory effects of gut GLP-1.[169]

GIP is involved in the regulation of glucose in the circulation by actin on islet of Langerhans to release insulin and glucagone.[170] However, different studies demostrated contradictory data on the role of GIP in human energy homeostasis.[171] GIP receptors are expressed on the adipocytes to promote lipid storage,

on neurons in the arcuate nucleus (ARC), dorsomedial nucleus, and paraventricular nucleus of the hypothalamus to control food intake.[172] An array of studies has shown the possible correlation between central GIP signaling, body weight gain and adiposity, through a possible induction of neural leptin resistance. GIP has also been shown to have a direct relation to obesity.[173]

3.3. CCK and lipids

CCK is released by I cells, which are a subgroup of the enteroendocrine cell family. It is secreted in the proximal small intestine because of protein and lipid ingestion. CCK is released following lipid ingestion through a G-protein-coupled receptor 40 (GPR40) mediated mechanism, while it is released because of protein ingestion through calcium-sensing receptor (CaSR).

After being released by ECCs, CCK enters the bloodstream and reaches a peak in concentration of between 6 and 15 *p*mol/L, about 90 to 120 minutes after eating, timing being influenced by fat and protein content of the chymus.[174] CCK is released as a pro-hormone, or pro-CCK, which is then cleaved and activated by prohormone that converts pro-CCK into various forms giving rise to different types of CCK.[175] For instance, CCK8 is the most abundantly utilized form, followed by other types such as CCK58, CCK33 and CCK58, each acting at a different site along the gastrointestinal tract.[176] Different studies have demonstrated the ability of CCK to activate vagal afferent pathways, showing for instance that CCK receptor 1 activation increased cAMP/PKA pathway, which is crucial for CCK activation of central afferent terminals. Furthermore, the activation of CCK receptor1/cAMP/PKA signaling in the small intestine eventually results into vagal afferent firing.[177]

Gut microbiota can affect lipid metabolism acting on bile acids, cholesterol and lipoprotein production. Some bacteria can produce enzymes to deconjugate bile acids, modifying their enterohepatic circulation and altering cholesterol levels.[178] Lipids can increase CCK release and CCK receptor antagonists are used as therapeutic targets to reduce the satiety senses after the intestinal passage of lipid in both humans and experimental models.[179,180] In detail, lipids promote the secretion of CCK and activate VANs to decrease food intake maybe be due to chylomicron formation and activation of a sensory mechanism on the basolateral region of EECs.[181,182] In addition, Pluronic L-81, that can block chylomicron formation, significantly reduces the anorexigenic effects of lipid administration decreasing CCK release, celiac and cervical vagal afferent activation, which are typical effects after lipid infusion.[183,184]

In summary, EECs in the GI tract can secrete different gut peptides affecting energy and glucose homeostasis. Overall, there is strong evidence that suggests how CCK can impact on energy and glucose homeostasis through the activation of a gut-brain vagal pathway, and more studies are required to analyze the mechanism of the process.[185]

4. INVOLVEMENT OF FERROPTOSIS IN THE GUT-LIVER-BRAIN AXIS

Iron dysregulation and ferroptosis have been shown to be active players in the progression of different neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS), multiple sclerosis (MS) and PD.[186-188-189] In neurodegeneration, multiple features of ferroptosis are reported, such as accumulation of lipid peroxidation products, depletion of glutathione, excess of extracellular glutamate and increased lipoxygenase (LOX) activity.[190,191] Microglia are sensitive to iron overload-induced ferroptosis and clinical investigations suggest how ferroptosis inhibitors may represent a therapeutic approach in these types of diseases. In fact, in a human induced pluripotent stem cell-derived tri-culture system containing neurons, astrocytes and microglia, the latter showed the highest transcriptional response to iron accumulation identifying a subgroup of microglia with a distinct ferroptosis-associated transcriptomic signature (FAS). The removal of microglia in the culture system decreased neuronal lipid peroxidation and reduced the death of neurons.[192] Microglia may induce ferroptosis in neurons through the production of pro-inflammatory cytokines, such as IL-1β, IL-6 and IL-8.[193] Microglial uptake of iron may be protective at the beginning of the disease, while a neurotoxic state is later established and leads to the cellular damage. In addition, several data strongly support the concept that the dying microglia may release factors that escalate neuronal death. Moreover, it has been demonstrated that increased endogenous levels of α-syn oligomers are correlated with high cytosolic calcium influx by changes in plasmalemmal membrane potential or activation of glutamate receptor.[194] In the absence of lipid peroxidation, the α-syn-induced calcium dysregulation is also abolished, and physiological calcium signalling is restored.[195]

On these bases, ferroptosis can induce and promote diseases by affecting the GLA and the interaction among these organs, microbiota and environmental factors. For example, the imbalance of intestinal microbiota can enhance microbial metabolites, such as SCFAs, and gut permeability, thereby inducing inflammatory

response and NAFLD.[196] The involvement of ferroptosis in NAFLD is mainly linked to the increase of lipid peroxidation. Moreover, the most important signaling pathways may incorporate Nrf2- GPX4 and AKT-GSK3β-Nrf2 pathways.[197] These imbalances of gut microbiota can be modulated by *Pleurotus geesteranus* polysaccharides through the reduction of oxidative stress acting on Nrf2/HO-1 and TLR4/NF-kB signaling pathways with the result to protect the intestinal barrier.[198] Furthermore, ferroptosis can mediate liver-brain axis affecting the hepatic metabolism level of amino acids and causing neuroinflammation through AKT/Nrf2/GPX4 and Nrf2-ARE pathways leading to ROS production.[199] Nrf2 can block the process of ferroptosis by its nuclear translocation and production of proteins related to iron metabolism, such as ferritin, ferroportin and SLC7A11.[200]

In summary, ferroptosis may modulate the gut-brain axis influencing the progression of the neurodegenerative disorders, acting on two main targets: Nrf2 and HO-1.[201,202] Nrf2 play a crucial role in the preservation of epithelial tight junction and gut barrier,[203,204] whereas, HO-1 represents an important factor regulating the antioxidant and neuro-protective responses.[205] The deposition of iron, GPX4 inactivation and lipid peroxidation accumulation cause damage of cell membrane, cellular junctions and subcellular organelles that contribute to: (i) the pathogenesis of IBD, (ii) initiate and continue liver injury, and (iii) to induce neurodegenerative disease onset mainly reaching the brainstem through the hepatic afferent vagus nerve.[206,207] For all these reasons, iron chelators represent an important approach for the process of ferroptosis, but many of them may have difficulties in the progression along the GLBA and may disrupt homeostatic redox functions.[208] However, some preclinical studies have used lipid peroxidation inhibitors targeting lipid peroxidation and oxidative stress, such as vitamin E derivative vatiquinone acid and activators of the antioxidant NRF2 pathway.[209]

4.1. Specific neuronal territories as main targets of ferroptosis and neurodegeneration along the gut-liver-brain axis

Ferroptosis has been demonstrated to be an important factor in the development of pathological processes in the brain. This is arguably secondary to the relatively higher susceptibility of the CNS to iron-induced lipid peroxidation, because of an intrinsic increased energy consumption and lipid content, as well as lower tolerance to ROS.[210]

Specifically, hippocampal neurons show increased levels of mitochondrial ferroptosis following general anesthesia, partially explaining the mechanism of anesthesia-induced ischemia-reperfusion injury.[211] As isoflurane is often used in general anesthesia, ferroptosis inhibitors and mitochondrial activators (such as ferrostatin-1 and dimethyl fumarate, respectively) could be used to decrease hippocampal susceptibility to ferroptosis.[212] Moreover, ferroptosis significantly induces memory decline. Very interestingly, this seems to be slowed down by hormone-replacement therapy in post-menopausal women,[213] possibly via anti-neuroinflammation and anti-oxidative stress mechanisms.[214]

The importance of ferroptosis in neurological diseases is underlined by the potential treatment strategies that can be utilized in future. For instance, in the hippocampus of animal model experiments mimicking diabetes-related cognitive dysfunction, *sinomenine* showed a neuroprotective potential against erastin-induced ferroptosis via the GBA[215] and by increasing EGF expression (known to reduce oxidative stress via the Nrf2/HO-1 signaling pathway).[216] Moreover, another study showed the potential to slow down diabetes-induced cognitive impairment by inhibition of ferroptosis via activation of AMPK.[217] The hippocampus is also highly susceptible to sepsis-associated encephalopathy (SAE), secondarily to ROS formation and altered ferroptosis-related protein expressions[218]. Similarly, ferroptosis has been shown to have a causative effect in traumatic brain injury (TBI)[219] as well as hemorrhagic and ischemic strokes.[220,221] Reduction of ferroptosis and improved mitochondrial dysfunction were achieved by administration of irisin,[218] a molecule proven to be a potential therapeutic target for cerebral ischemia,[222] neurodegenerative diseases[223] and TBI,[224] through its anti-oxidative, anti-inflammatory and anti-ferroptotic activities. Interestingly, also low-dose acetaminophen prevents hippocampal ferroptosis in septic mice via the GPX4 and FSP1 pathways.[225] In the hippocampus, ferroptosis is significantly involved in AD,[226,227] opening new potential diagnostic and therapeutic possibilities against the most prevalent neurodegenerative disease in the general population.[228]

Moreover, putamen and globus pallidus are particularly susceptible to iron accumulation in PD[229] and in other diseases that involve iron accumulation in the brain.[230] Progressive loss of spontaneous activity in the putamen also contributes to impaired task performance in patients with PD.[231] In a metanalysis of post-mortem measurements of iron levels in the brain of PD patients, significantly high levels of iron deposition were observed in the whole Basal Ganglia system, including the substantia nigra, the caudate nucleus and the globus pallidus, suggesting a crucial role of ferroptosis in the pathogenesis of PD.[232] Ferroptosis plays a role also in the development of Huntington's disease (HD), an inher-

ited, neurodegenerative disorder. This neuro-disorder involves striatum, and it is characterized by the gradual development of choreic movements. Studies suggested high levels of iron in HD patients.[233,234] Unsurprisingly, ferrostatin-1 showed the potential to prevent iron-dependent oxidative stress and the capacity to reverse decrease neuronal cell death in cellular models of HD.[235] Moreover, laduviglusib, a highly selective inhibitor of Glycogen synthase kinase 3, was demonstrated to target ferroptosis-related genes in striatal neurons in the setting of HD.[236]

Doublecortin deficiency and the presence of ferroptosis were observed in a post-mortem analysis of the caudate nucleus of AD patients.[237] Also, the nigrostriatal system can be specifically pathologically affected by ferroptosis. This can be perhaps exemplified by the effect of methamphetamine (the second most used recreational drug, that works through an amphetamine-type neuronal stimulation),[238] whose use was associated to increased iron deposits in both the substantia nigra and caudate nucleus. Very interestingly, the administration of iron chelators in the setting of methamphetamines use proved to decrease the pathological nigrostriatal changes via attenuated iron deposition and ROS formation, as well as dopaminergic cell death.[239]

Several studies have demonstrated that neuroinflammation, or rather the activation of the neuroimmune cells into proinflammatory states, is implicated in neurodegenerative diseases, such as AD, Synucleinopathies, ALS and HD not only because of neurodegeneration but also as a pivotal player in this process.[240] In this context, CNS inflammatory response is orchestrated by an interaction of microglial cells, infiltrating myeloid cells, astrocytes, the BBB cells (endothelial cells, pericytes, astrocytes) and the action of signaling molecules (cytokines, chemokines, and growth factors), which produce both central and peripheral reaction.[241]

Interestingly, the process of neuroinflammation could be triggered and highlighted by both gut and liver produced molecules as well as by ferroptosis.[242–244] Therefore, ferroptosis contributes to the connections of the gut-liver-brain axis, as well as to the interplay between neuroinflammation and neurodegeneration in the context of different brain areas.

CONCLUDING REMARKS

Ferroptosis, an iron-dependent form of programmed cell death, is emerging as a critical factor in neurodegeneration, intricately linked with neuroinflammation and the GLBA. The process of ferroptosis is characterized by the accumulation of lethal lipid peroxides, leading to cell death and dysfunction. In neurodegenerative diseases like AD and PD,[245–247] this pathway is significantly activated, contributing to the degeneration of vulnerable neuronal populations.

Neuroinflammation, characterized by the activation of the brain's immune cells and the release of proinflammatory cytokines, exacerbates ferroptotic mechanisms. The inflammatory milieu enhances oxidative stress, fuelling iron accumulation and lipid peroxidation,[248] thereby contributing to neuronal death. This implies a vicious cycle where neuroinflammation and ferroptosis amplify each other.

Furthermore, the GLBA plays a substantial role in modulating these interactions. The gut microbiota influences systemic inflammation and oxidative stress through the production of metabolites and the modulation of immune responses. Dysbiosis can lead to increased gut permeability, allowing inflammatory mediators to enter circulation and reach the brain,[249,250] thereby directly affecting neuroinflammatory states and potentially triggering ferroptotic pathways.[250,251]

The liver also contributes to this axis, as it regulates systemic iron metabolism and inflammatory responses. Liver dysfunction can lead to altered iron homeostasis and increased production of inflammatory mediators, both of which can influence ferroptotic activity in the brain.[252]

This interplay suggests that targeting the GLBA could potentially offer therapeutic strategies to mitigate ferroptosis and neuroinflammation. By maintaining gut microbiota balance and ensuring proper liver function, it may be possible to modulate systemic inflammation and oxidative stress, thus reducing neuronal vulnerability to ferroptosis and slowing the progression of different neurodegenerative disorders. Understanding these complex interactions underscores the need for integrated therapeutic approaches that address the multifaceted nature of GLBA.

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