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The Neurobiology of Hepatic Encephalopathy

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Abstract

Despite significant recent breakthroughs, with rapid discoveries provided by the twentieth century, hepatic encephalopathy remains an ancestral enigma that accompanies the history of mankind. Much of this is due to the reductionist view that a single process would have primacy over others, with the emphasis on hyperammonemic theory being its greatest example. Since other factors, such as the intestinal microbiota composition, the synergism with neuroinflammation, and the role of glutamatergic and GABAergic tonus balance have been discovered, it has become clear that the traditional and linear view of scientific research allows the understanding of the initial state of multiple dysfunctional systems, but is unable to predict the overall behavior of the disease. As consequence, there is a lack of innovative interventions for controlled clinical trials, making its therapeutic management very limited. The objective of this chapter is to provide a general theoretical overview of the most relevant hypotheses and findings in the neurobiology of hepatic encephalopathy, and how its toxic, metabolic and immunological alterations affect the cellular metabolism and neurotransmission dynamics, causing its characteristic cognitive and motor manifestations.

Keywords: cirrhosis, hepatic encephalopathy, cognition, minimal hepatic encephalopathy, motor, neurotransmission

1. Introduction

Since ancient Babylonian times (1894–1595 B.C.), people have been aware of the influence of liver dysfunction on cognition [1]. In the Ancient Orient, the liver was considered the center of life and mental activity. Hippocrates (460–370 B.C.) and Celsus (25 B.C.–50 A.D.) were pioneers in the description of behavioral disorders associated with the hepatic failure. In the *Corpus Hippocraticum*, there is the report of a patient with jaundice who “barked like a dog, could not be contained, and said nothing understandable” [2]. Galenus (129–199 A.D.), physician of the Roman centurions, considered the liver responsible, alongside the heart and the brain, for the triple control of the natural, animal and vital spirits. In his theory, he imagined that these spirits were derived from food processing and routed through the bloodstream to the cerebral ventricles [3]. In the Modern Age, especially in the eighteenth century, several records of neuropsychiatric disorders in cirrhotic patients have been described. It is from that time that Giovanni Battista Morgagni (1682–1771 A.D.) detailed the progressive nature of the disease in the famous *De Sedibus et Causis*

Morborum Per Anatomen Indagatis (1761). In the Contemporary Age, Friedrich Theodor von Frerichs (1819–1885 A.D.) carried out an extensive documentation of the cognitive and motor changes found in cirrhosis [2]. In the twentieth century, especially since the 1930s, several publications have enumerated the typical alterations in the disorder known as hepatic encephalopathy, with particular emphasis on the hypothesis that its pathophysiology would be caused, in some way, by the reduction of ammonia clearance produced in the gut [4].

The mechanisms of hepatic encephalopathy, however, remain far from being fully elucidated. No significant breakthrough occurred simultaneously in clinical and basic research in the second half of the twentieth century. Indeed, up to the present moment, in the twenty-first century, it seems unlikely that any new paradigm will emerge in a short term. In consequence, there is a lack of innovative interventions for controlled clinical trials, making its therapeutic management very limited [5].

The American and European Associations for the Study of the Liver (AASLD and EASL) define hepatic encephalopathy as “a brain dysfunction caused by liver insufficiency and/or portosystemic shunt” and add that “it manifests as a wide spectrum of neurological or psychiatric abnormalities ranging from subclinical alterations to coma” [6]. Such a definition encompasses the need for detection, quantification, and differentiation of other conditions that affect cognition, regardless of insufficiency and shunt. Unfortunately, little attention has been paid to the importance of the differential diagnosis of secondary causes of cognitive deficits in patients with cirrhosis [5]. In the practice of a reference unit in Brazil, 84% of the studied population had a concomitant condition that justified or aggravated the cognitive dysfunction, such as interferon use, major psychiatric illness (mainly depression), diabetes mellitus, neoplastic disease, use of psychotropic drugs, hypothyroidism, visual impairment, use of illicit drugs, chronic obstructive pulmonary disease, heart failure, HIV seropositivity, and vitamin B12 deficiency [7].

Approximately 30–50% of patients with chronic liver diseases, such as cirrhosis, have minimal hepatic encephalopathy, with decreased information processing speed, attention deficits, and motor incoordination. There is evidence that even minimal cognitive deficits can have a major impact on quality of life, with decreased learning and driving ability, as well as increased caregiver overload [5]. The 2014 Practice Guideline on Hepatic Encephalopathy describes minimal hepatic encephalopathy as a condition in which there are “psychometric or neuropsychological alterations of tests exploring psychomotor speed/executive functions or neurophysiological alterations without clinical evidence of mental change” [6]. This definition has a primary requisite that patients do not present any clinically evident manifestations of cerebral dysfunction in the clinical evaluation. The Guideline Development Group suggests that the operational criterion for the diagnosis of this condition should be “abnormal results of psychometric and neuropsychological tests without any clinical manifestations”, although it is clear that there are no universal diagnostic criteria and that, therefore, local testing standards are necessary [8].

To overcome all difficulties related to the understanding of hepatic encephalopathy, it is essential to establish a common language among the several research areas related to the disease. The aim of this chapter is to provide a general theoretical overview of the most relevant hypotheses and findings in the neurobiology of hepatic encephalopathy, in order to contribute to the construction of an integrated approach to the subject.

2. The role of intestinal microbiota and enterocytes

Since the 1930s, ammonia has been known to play an important role in the pathophysiology of hepatic encephalopathy [4]. However, hyperammonemia can be

found in patients without hepatic encephalopathy, and normal levels of ammonia can be seen in patients with advanced hepatic encephalopathy [9]. Serum ammonia dosage is also not a good parameter for evaluating the severity of the disease [10]. In addition, studies have demonstrated that hyperammonemia is not a sufficient condition to produce cognitive deficits in minimal hepatic encephalopathy [11].

Ammonia is produced in the body from the metabolism of intermediate amino acids, and its concentration is increased by the action of intestinal bacteria. In adults, approximately 1000 mmol (17 g) of ammonia is produced per day [12]. In cirrhotics, its serum concentration increases two to three times, an increase that is also exacerbated by the induction of glutaminase expression by enterocytes, which hydrolyzes the amino acid glutamine into glutamate and ammonia to obtain energy [9]. At least one haplotype of the glutaminase gene appears to be related to a higher propensity to develop clinically symptomatic encephalopathy, demonstrating that the constitutive activity of this enzyme undergoes genetic variations [13].

The small and large intestines are colonized by a massive variety of microorganisms, collectively known as microbiota. About two-thirds of the gut microbiota is unique to each individual, being composed of more than a thousand species of bacteria, although less than 170 commensals predominate, such as *Bacteroides* and *Firmicutes* [14]. Some studies have shown that the composition of the intestinal microbiota affects the severity of hepatic encephalopathy by modulating its toxicological profile [15].

Recently, the concept of intestinal dysbiosis has been highlighted as a risk factor for the development of hepatic encephalopathy [5]. It refers to changes in bacterial composition, with a decrease in the rate of potentially beneficial autochthons and an increase in the rate of pathogens such as *Staphylococcaceae*, *Enterobacteriaceae*, and *Enterococcaceae* [9]. Such alterations potentiate ammonia synthesis and a proinflammatory systemic environment, contributing to neuroinflammation [14]. One of the major obstacles in assessing the impact of these changes, however, is that the composition of the microbiota varies according to geographic differences, making it practically impossible to compare individuals from different cultures and environments [9].

The use of non-absorbable disaccharides (e.g., lactulose and lactitol) remains the mainstay for the treatment and secondary prevention of hepatic encephalopathy. Although widely known for their laxative properties and their capacity to inhibit glutaminase activity, they have the ability to modify positively the intestinal microbiota, inducing the growth of commensal microorganisms. The 2014 Guideline on hepatic encephalopathy does not recommend its use for the treatment of minimal hepatic encephalopathy, but states that exceptions can be made on a case-by-case basis if there is impairment in driving ability, work performance, or quality of life [16].

3. The role of hepatocytes and endothelial cells

Ammonia reaches the liver through the portal circulation and is purified by periportal hepatocytes, which incorporate it into urea synthesis, or by perivenular hepatocytes, which catalyze the condensation of glutamate and ammonia into glutamine by the action of glutamine synthetase [9]. The ammonia concentration in the portal vein ranges from 300 to 600 μmol , dropping to 20–60 μmol in the hepatic veins [12]. The liver, thus, plays a central role in the regulation of its levels and, in healthy individuals, removes it almost completely: small amounts of escaping ammonia are metabolized in the skeletal muscle (which also expresses glutamine synthetase), and in the kidneys (where more than 70% of it is reabsorbed). In case of hepatic failure and portosystemic shunt, ammonia escapes this detoxification process, increasing its serum concentration [9]. This leads the skeletal muscle to play an important role

in its clearance, but this metabolic pathway is not sufficient to eliminate it from the body and there is a loss of muscle mass in about 40–76% of those with cirrhosis [17]. Moreover, it is common for such patients to have concomitant zinc deficiency, an important cofactor for glutamine synthetase, which may aggravate its elimination [9].

In cirrhosis, hepatic gluconeogenesis is impaired. The amino acid precursors of glucose synthesis, such as alanine, threonine, glycine, and aspartate, are increased, whereas peripheral anaerobic glycolysis increases lactate and pyruvate levels [18]. Of particular importance, studies demonstrate that glycine may be an ammoniagenic amino acid, causing increased ammonia synthesis in the gut and brain through induction of a reaction mediated by glycine oxidase [19]. This has been explored as a potential therapeutic target, since the reaction is bi-directional and the removal of glycine can lead to the use of ammonia to replenish its stocks, lowering its levels [20].

On the other hand, the low systemic availability of glucose causes hepatocytes to produce more ketone bodies from fatty acids, for the energetic metabolism of nervous and muscular tissues. However, it is hypothesized that in situations like this, hepatocytes prioritize the production of energy for its own subsistence rather than synthesizing products destined for exportation to other tissues [18]. Thus, ketogenesis would also be impaired, which is corroborated by significantly decreased beta-hydroxybutyrate and acetoacetate levels, resulting in a precarious energy metabolism in the central nervous system in the advanced stages of the disease [18, 21].

Given its location and abundant vascular supply, with immense exposure to antigens absorbed by the intestine, the liver regulates important immune functions [9]. In cirrhosis, intestinal bacterial overgrowth associated with hepatocellular failure triggers a systemic immune reaction, bypassing endotoxins such as membrane lipopolysaccharides, flagellins, and peptidoglycans for arterial circulation [15, 22]. Circulating cytokines, such as tumor necrosis factor alpha (TNF α), interleukin 1b (IL-1b) and interleukin 6, induce the synthesis of nitric oxide and prostanoids in endothelial cells, triggering a state of inflammatory hyperemia that facilitates the uptake of ammonia by the central nervous system [9]. In addition, the proinflammatory cytokines generated by the vascular endothelium activate the cells of the immune system in the brain parenchyma and the microglia, contributing indirectly to neuroinflammation [11].

4. The role of astrocytes

Astrocytes are part of the blood-brain barrier and protect neurons from the toxic effects of ammonia [12]. Its perivascular extensions are rich in aquaporin 4, a protein constituent of water channels. Astrocytes are among the cells with the highest glycolytic activity of the central nervous system and are estimated to be responsible for 30% of its metabolism. They are believed to be particularly susceptible to the development of edema because they are part of the glymphatic system [23], a paravascular system discovered in 2012, which receives continuous influx of periarial cerebrospinal fluid and has a leakage network through the perivascular spaces into the cerebral veins [24].

Liver failure can result in an uncontrollable rise in ammonia levels, which penetrate virtually all organs. Although the central nervous system is partially protected by the blood-brain barrier, which remains relatively intact until advanced stages of the disease, excessive amounts of ammonia can overtake it [12, 25]. Therefore, concentrations that normally range from 0.2 to 0.3 μmol in normal subjects can reach the mark of 3 to 5 mmol in patients with hepatic encephalopathy [4]. However, along with perivenular hepatocytes and skeletal muscle, astrocytes express glutamine synthetase and have the ability to convert ammonia into glutamine [4, 9].

The accumulation of glutamine in astrocytes, although not directly toxic, drastically affects its functioning [26]. Firstly, glutamine has an osmotic action, inducing predominantly cytotoxic and slightly vasogenic edema [25]. Generally, any form of edema increases the distance for diffusion of oxygen and metabolites in the brain parenchyma, exposing microareas of borderline irrigation to hypoxia [23]. This phenomenon is more pronounced in acute hepatic failure, in which the counterregulatory mechanisms do not have time to act, but can also be detected in the magnetic resonance imaging (MRI) of patients with chronic liver failure [9, 26]. Secondly, exceeding glutamine is transported to the mitochondria, where, by glutaminase action, it is hydrolyzed back into glutamate and ammonia. The passage of the latter to the interior of the mitochondria causes oxidative stress and modifies the internal mitochondrial membrane diffusivity, through the opening of permeability transition pore, causing water accumulation in the mitochondrial matrix, low capacity of oxidative phosphorylation, and low adenosine triphosphate (ATP) production [11, 12]. This results in a vicious cycle of formation of reactive oxygen and nitrogen species (free radicals) with mitochondrial damage [9].

Studies with cultures of astrocytes and neurons show that only the former increase the production of free radicals when exposed to glutamine [12] and that's why astrocytes can be considered the basic morphofunctional unit of hepatic encephalopathy: the histopathological milestone of the disease is the swelling of astrocytes, both in the cytoplasm and in the nucleus, with chromatin marginalization, prominent nucleoli and glycogen accumulation, accompanied by little neuronal alteration [4, 27].

The effects of chronic hyperammonemia and astrocytic edema can be verified in specific sequences of brain MRI. In the spectroscopy of the basal ganglia, the Glx/ creatine ratio is increased and myo-inositol/ creatine and choline/ creatine ratios are decreased [14, 22]. Creatine is a constitutive marker of neurons and astrocytes. The increase of Glx demonstrates the accumulation of glutamine and glutamate [22]. This increase, however, seems to present large interindividual variations, and within a same animal model, there are forms in which there is a gradual increase, a strong increase followed by a plateau or only by a late rise [21]. Choline is a marker for the turnover of membrane phospholipids, and its decrease reflects reduction of basal metabolism of neurons and glial cells [22]. Furthermore, due to the osmotic imbalance generated by the accumulation of glutamine and glutamate, astrocytes export choline and myo-inositol, its main osmolyte, to the extracellular space, which leads to a reduction in the levels of the later, in an attempt to counterbalance the intracellular edema. This mechanism is known as regulatory volume decrease [11, 21, 23]. The diffusion-weighted imaging, in turn, shows interstitial edema resulting from the exportation of osmolytes from the astrocytes into the extracellular space, both in the white and gray matters. Because of that, multiple sites in the brain have an increase in mean diffusivity, including the frontal, temporal, inferior parietal, and insular lobes, as well as the corpus callosum, putamen, thalamus, and pons [22, 27]. The diffusion of water molecules, however, is not free; it reflects interactions with macromolecules, fibers, and membranes. It is important to emphasize that the diffusion-weighted sequences only show changes in the intra- and extracellular volume, and do not allow a definitive conclusion about the total amount of water present in the brain parenchyma [23].

5. The role of microglial cells

The activity of astrocytes and neurons can be modulated by microglia. The microglial cells are innate of the immune system, have phagocytic function and perform active surveillance of the brain parenchyma. In the absence of inflammatory stimuli,

they remain quiescent and have an aspect endowed with ramifications (resting phenotype). When an inflammatory stimulus occurs, they become reactive and acquire an ameboid aspect (active phenotype), migrating to the injured site, where they proliferate and produce neurotoxic and neurotrophic factors that control tissue damage and regeneration. In hepatic encephalopathy, molecules such as ammonia, glutamate, and some locally produced neuroactive steroids (neurosteroids) may trigger the transition from the resting phenotype to the active phenotype [11].

Neuroinflammation modulates glutamatergic activity. Studies have shown that microglial activation in the cerebellum of rats exposed to chronic hyperammonemia promotes an increase in the production of proinflammatory cytokines, such as $\text{TNF}\alpha$ and $\text{IL-1}\beta$, in addition to an increase in the expression of $\text{TNF}\alpha$ receptors. Of particular importance, $\text{TNF}\alpha$ receptors are also expressed on the surface of astrocytes and their stimulation induces increased glutaminase, contributing to the increase of glutamate synthesis [28]. There is also evidence that excess glutamate causes microglial activation, resulting in an intercellular vicious cycle [11].

Another important neurotransmission system affected by neuroinflammation includes a class of peripheral gamma-aminobutyric acid (GABA) receptor, known as translocator protein (TSPO), which is expressed in the outer mitochondrial membrane of neurons. Although poorly present under normal conditions, microglial activation strongly increases its concentration, which can be seen in cirrhotic patients through studies with positron emission tomography and carbon 11-labeled radiotracer that specifically bind to it [11]. It is known that TSPO mediates the synthesis of neurosteroids from cholesterol, and its increased expression provides an important link between neuroinflammation and increased GABAergic activity [12].

Like hyperammonemia, neuroinflammation is not sufficient to produce minimal hepatic encephalopathy: evidence of this is the fact that microglial proliferation can also be found in cirrhosis without encephalopathy, suggesting that it plays a role much more associated with neuroprotection than production of tissue damage [11]. Current knowledge supports the theory that there is the necessity of the coexistence of hyperammonemia and neuroinflammation, interacting synergistically, for the occurrence of neuropsychiatric disorders [10, 26]. In addition, at least one experimental study demonstrates that it is possible to produce cognitive deficits with the combination of these two factors, even in the absence of underlying liver disease [11].

6. The role of neurons

Hepatic encephalopathy has traditionally been assumed to be a metabolic disorder that affects glial cells but maintains the neuronal architecture preserved. However, this belief is easily contradicted by the presence of neuronal loss in its most extreme form: hepatocerebral degeneration. Such disorder is characterized by chronic manifestations (ataxia, dysarthria, apraxia, and parkinsonian symptoms), often associated with repeated and prolonged episodes of hepatic encephalopathy. Its anatomopathological study demonstrates not only astrocytic changes, but also neuronal loss in the basal ganglia, cerebral cortex, and cerebellum [15].

Most patients who develop episodes of hepatic encephalopathy demonstrate some degree of brain injury. Studies have shown that previous episodes of hepatic encephalopathy are risk factors for the development of cognitive impairment, which persists even after hepatic transplantation. In MRI, these findings are related to the fall of N-acetylaspartate in spectroscopy, a marker of neuronal density. This loss may be greater in some brain areas, such as the basal ganglia, which are particularly sensitive to oxidative stress injury, which explains some of its more prominent clinical manifestations, such as movement disorders [15].

It is known that in normal individuals, nitric oxide acts as a retrograde neurotransmitter to the neurons, activating the guanylate cyclase, with consequent increase of the cyclic GMP (cGMP) and decrease of the intracellular influx of chlorine in the glycine receptors. The resulting electrochemical imbalance decreases the threshold of neuronal depolarization, facilitating the generation of action potentials, with subsequent intracellular influx of calcium through ionotropic channels, which amplifies the phosphorylating cascade of the calcium-calmodulin complex, in a process that culminates with learning [29].

Hyperammonemia induces an increased expression of nitric oxide synthase in astrocytes, promoting the formation of excessive amounts of nitric oxide, which diffuses into the extracellular environment. Prolonged hyperexposure of neurons adjacent to nitric oxide depletes the formation of cGMP, but the activity of nitric oxide synthase remains unchanged. The result is a high intraneuronal calcium influx and subsequent activation of NADPH oxidase, leading to the formation of superoxide. Superoxide and nitric oxide then combine to form the free radical peroxynitrite, in another vicious cycle that results in apoptosis [12]. In addition, neuronal ATP depletion is observed because of low nucleotide synthesis and high degradation rate, although its levels do not appear to correlate linearly with the concentration of glutamine and ammonia in the brain parenchyma [21].

Cyclic GMP also plays an important role in the reduction of neuroinflammation and microglial activation. It is known that this reduction is associated with an increase in the concentration of IL-1b and TNF α receptors [28]. The fact that chronic hyperammonemia promotes decreased cGMP production has been explored as a potential target for drug-based experimental treatments that increase the concentration of cGMP by inhibiting its degradation (e.g., sildenafil and zaprinast). One of the major obstacles to this strategy, however, is the fact that cGMP seems to act within narrow concentration limits, above which its accumulation becomes equally counterproductive to neuronal activity [29].

Under normal conditions, glutamine and glutamate synthesized by astrocytes are transferred to neurons, which internalize them via excitatory amino acid transporters 1 and 2 (EEAT1 and EEAT2). In neurons and astrocytes, the storage process of glutamate within presynaptic vesicles depends on the activity of vesicular glutamate transporters (VGLUT), which have three isoforms (VGLUT1–3). The VGLUT3 isoform, expressed mainly by astrocytes, is easier to release glutamate than the VGLUT1 and VGLUT2 isoforms found in neurons, which depend on intracellular calcium variations. That is the reason why astrocytes are more likely to release accumulated vesicular glutamate than neurons [4, 12]. Moreover, glutamate is able to donate amines for the synthesis of serine, a precursor amino acid of glycine, increasing its synthesis and, consequently, of ammonia in the brain parenchyma [19]. Hyperammonemia, on the other hand, reduces the expression of EEAT1 and 2 on the neuronal surface, impairing its capacity of uptake. The result is the extracellular accumulation of glutamate, with consequent hyperactivation of adjacent receptors. This sequence of events seems to be the key in the pathophysiology of hepatic encephalopathy [4, 12].

7. Effects on neural networks

Cognitive functions—attention, executive functions, memory, visuospatial skills, language, and social cognition—are the emerging results of neurotransmission [26]. They depend on the cooperation of multiple cortical areas, connected to each other through the white matter by bundles and fascicles of axonal fibers, in circuits known as neural networks. Changes in the synchronization of the activity

of these different regions contribute to the appearance of neurological deficits. This synchronization depends on the integrity of the white matter, which modulates the information processing speed [30].

During the progression of hepatic encephalopathy, the diffusion-weighted imaging on MRI demonstrates cumulative abnormalities in the white matter. In addition to interstitial edema, there may be macroscopic atrophy of the white matter and damage to the microstructural integrity of bundles and fascicles. Studies in patients with cirrhosis have shown that these changes correlate with the incidence of attention deficit, executive dysfunction, and increase in the number of falls [30]. The largest reductions appear to occur in the frontal white matter and in the globus pallidus [27]. In addition, cortical thickness decreases in several regions, such as the lateral superior temporal gyrus and the precuneus, which may also present correlations, respectively, with attention and visuospatial deficits [30].

The final result of the accumulation of toxic, metabolic, cellular, and immunological alterations produced by liver failure and portosystemic shunt is the occurrence of dysfunction in the main axes of neurotransmission [31]. It is important to emphasize, however, that a same system may be involved with more than one cognitive function and that the mechanisms that lead to cognitive impairment are different from those involved in motor impairment [26]. **Table 1** summarizes the main changes found in neurotransmission. The most known repercussions for each neural system will be discussed below.

Neurotransmission changes in hepatic encephalopathy		
Increased synthesis of neurotransmitters at presynaptic terminals	↑ Glutamate	[28]
	↑ Glycine	[19]
	↑ Histamine	[31]
Increased release of neurotransmitters at presynaptic terminals	↑ Glutamate (VGLUT3)	[4]
Decreased reuptake of neurotransmitters at presynaptic terminals	↑ Glutamate (↓ EEAT1 and 2)	[4]
	↑ GABA (reversal of GAT3)	[32]
Increased degradation of neurotransmitters in the synaptic cleft	↑ GABAergic modulatory neurosteroids	[33]
	↓ Acetylcholine (↑ acetylcholinesterase and butyrylcholinesterase)	[34]
	↓ Serotonin (↑ MAO-A)	[35]
Modulation of receptor activity at postsynaptic terminals	↑ GABAergic modulatory neurosteroids	[12, 26]
	↑ Activity of metabotropic and ionotropic glutamatergic receptors (AMPA)	[26]
	↓ Activity of adenosinergic receptors	[36]
Changes in signal transduction cascade at postsynaptic terminals	↑ Intracellular calcium	[12]
	↑ cGMP	[28]
Increased synthesis of retrograde neurotransmitters at postsynaptic terminals	↑ Nitric oxide	[29]

Table 1.
Main alterations found in neurotransmission in hepatic encephalopathy.

8. Effects on the glutamatergic system

Glutamate is the main excitatory neurotransmitter of the central nervous system [31]. Two glutamatergic circuits are particularly important in the pathophysiology of hepatic encephalopathy: (1) an yet unproven hypothetic pathway that would descend from the frontal lobe and (2) the perforant pathway originated in the entorhinal cortex.

It is believed that the frontal descending pathway (**Figure 1**) originates in layer V pyramidal neurons and projects to the centers of other neurotransmitters in the brainstem. There, it performs synapses with dopaminergic neurons of the ventral tegmental area and the substantia nigra, the serotonergic neurons of raphe nuclei and noradrenergic neurons of the locus coeruleus, influencing their activity [37]. If this hypothesis is correct, glutamatergic hyperactivity would act as a final pathway common to the changes induced by hyperammonemia and neuroinflammation, disturbing other neurotransmission systems, in steps that would invoke neuropsychiatric symptoms, and, in more severe cases, cause coma [4]. In addition, the frontal descending pathway would act as a “brake” for the dopaminergic pathway that leaves the ventral tegmental area toward the accumbens nucleus (located between the putamen and the caudate nucleus), influencing its activity through inhibitory GABAergic interneurons in the brainstem. This would result in tonic inhibition of dopamine release, with important consequences for executive and motor functions [37].

The perforant pathway (**Figure 2**) originates in the medial portion of the temporal cortex, called the entorhinal cortex, and projects to the granular cells of the dentate gyrus. The axons of these cells form a pathway of mossy fibers, which goes to the *Cornu Ammonis* (CA) or Ammon’s horn, more precisely to the pyramidal cells of the CA3 region. Then, the pyramidal cells emit excitatory collaterals, the Schaffer collaterals, that go to the pyramidal cells of the CA1 region. A brief discharge of high-frequency stimuli in any of these three components of the perforant pathway increases the excitatory postsynaptic potentials in hippocampal neurons, which can last for hours, days, or even weeks. This facilitation is called long-term potentiation and, in addition to the hippocampus, also occurs in the amygdala, striatum (putamen and caudate nucleus), and cerebellar Purkinje cells, being essential for the formation of new traces of memory and learning [29, 32].

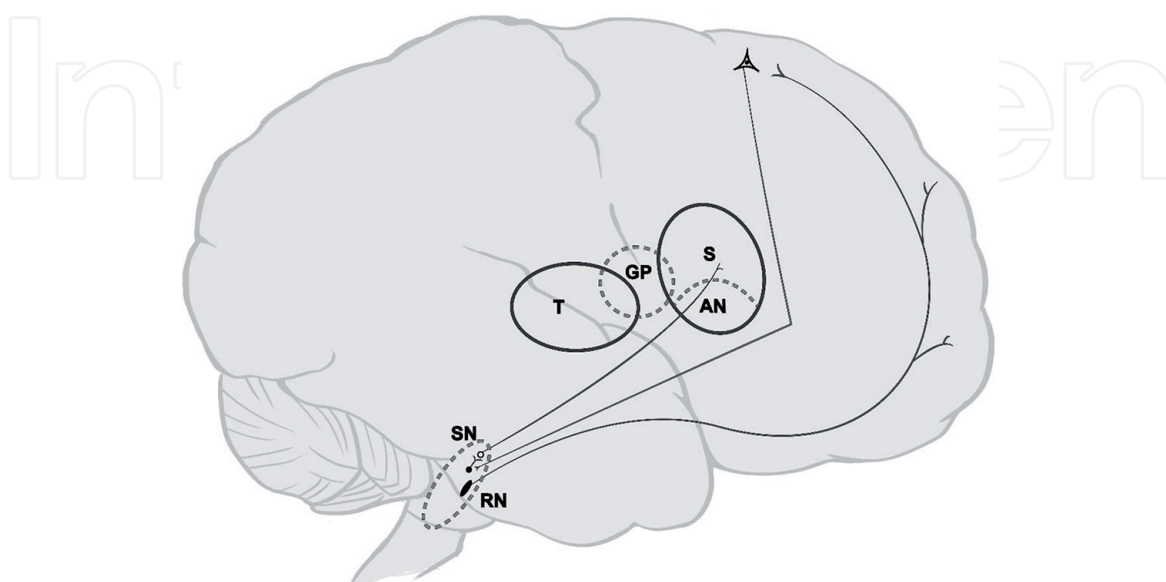


Figure 1.
 The frontal descending pathway would originate in the frontal cortex and influence directly or indirectly (through inhibitory interneurons) the activity of the neurotransmitter centers of the brainstem. AN: accumbens nucleus, GP: globus pallidus, RN: raphe nucleus, S: striatum, SN: substantia nigra, and T: thalamus.

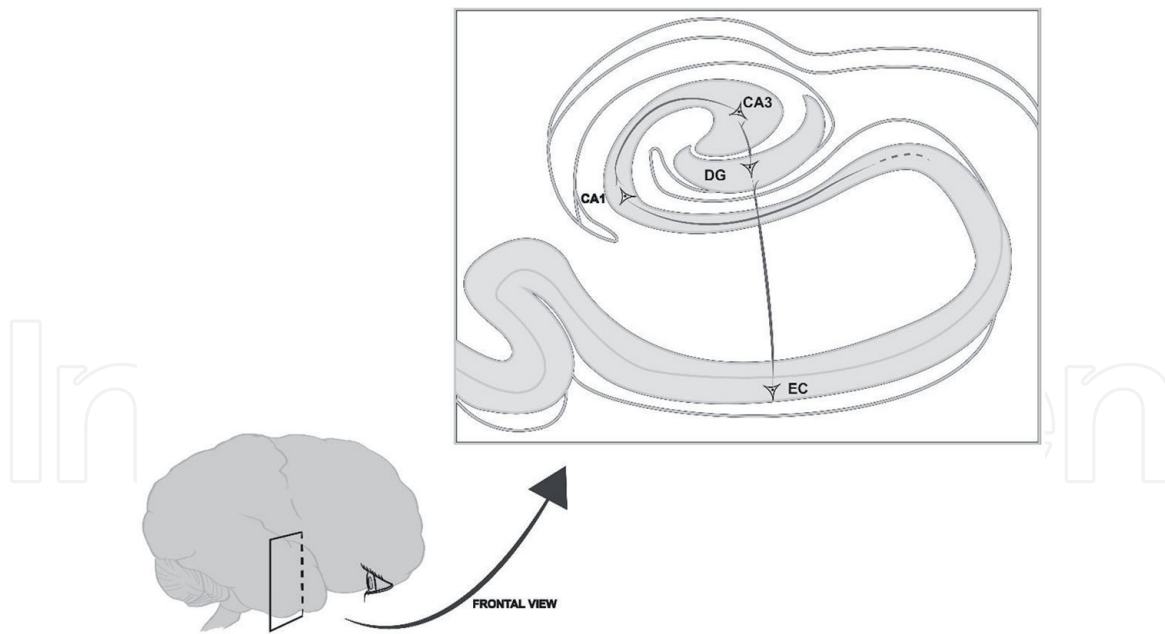


Figure 2.

The perforant pathway originates in the entorhinal cortex (EC) and extends to the dentate gyrus (DG), from which neurons establish synapses with the CA3 and CA1 regions of the hippocampus, being involved with memory formation.

Glutamate receptors are classified as metabotropic (coupled to G protein) and ionotropic (bound to ion channels). There are at least eight subtypes of metabotropic receptors and three classes of ionotropic receptors named according to agonists that selectively bind to them: NMDA (N-methyl-D-aspartate), AMPA (α -hydroxy-5-methyl-4-isoxazolepropionic acid), and kainate [37]. The first two have a particular relevance in hepatic encephalopathy, since the accumulation of glutamate in the synaptic clefts causes its hyperactivation, with excessive calcium influx [12]. This constant opening (tonic) of the ionotropic channels results in greater production of free radicals, with consequent neuronal apoptosis [26, 31]. The development of this process in the perforant pathway is a possible explanation for the episodic memory deficits presented by cirrhotics [12, 32]. Ammonia also induces apoptosis as a result of overproduction of nitric oxide [12], and this could explain why in some individuals such deficits become irreversible.

9. Effects on the GABAergic system

Cortical neurons are also modulated by GABA-secreting neighboring interneurons, the main inhibitory neurotransmitter of the central nervous system [31]. Such cells organize themselves so that they can project their axons directly onto pyramidal cells, inhibiting glutamatergic neurotransmission, or extending their axons to other GABAergic interneurons that influence pyramidal cells, inhibiting the inhibition (and therefore, disinhibiting) of glutamatergic activity.

There are three main types (GABA_A , GABA_B , and GABA_C) and numerous subtypes of GABA receptors. GABA_A and GABA_C receptors are ionic channels sensitive to ligands and are part of a macromolecular complex that forms an inhibitory chlorine channel, whereas GABA_B receptors are members of a different class, bound to protein G (metabotropic receptors). Depending on the composition of their subunits, GABA_A receptors may be sensitive to benzodiazepines [37]. Nonbenzodiazepine-sensitive subtypes are located outside the synapses, capturing not only GABA that diffuses beyond it but also locally released neuroactive steroids as a consequence of microglial

activation [31]. Nonbenzodiazepine-sensitive extrasynaptic GABA_A receptors promote tonic inhibition of postsynaptic neurons, as opposed to phasic inhibition induced by benzodiazepine-sensitive GABA_A receptors. In addition, GABA_A receptors bind effectively to other modulators, such as alcohol and neurosteroids, in a different location than GABA agonists, the so-called allosteric sites [37].

Experimental studies have shown that, in chronic hepatic encephalopathy, increased GABAergic tone in the cerebellar cortex results in motor incoordination [28, 32]. Several theories have been proposed throughout the history to explain the elevation of the activity of this neurotransmission pathway: (1) increased GABA synthesis, (2) increased expression of GABA_A receptors in postsynaptic terminals, (3) modulation of GABA_A receptors by neuroactive steroids, and (4) reversion of the action of astrocytic GABA transporters [26, 28]. Most studies, however, show with confidence that: (1) although glutamine is a precursor for GABA, GABA synthesis is not increased in hepatic encephalopathy and (2) GABA_A receptor expression does not change in chronic liver insufficiency [12, 21, 26]. Therefore, hypotheses (3) and (4) regarding the modulation of GABA_A receptors by neurosteroids and reversion of the action of astrocytic transporters are those that require greater considerations.

Experimental studies with acute hepatic failure demonstrate that neurosteroids synthesized locally by microglial cells from cholesterol participate in the modulation of GABA_A receptor activity. Such neuroactive steroids may have an inhibitory effect (e.g., pregnenolone), functioning as positive allosteric modulators of GABA_A, or excitatory receptors (e.g., allopregnanolone and tetrahydrodeoxycorticosterone), functioning as negative allosteric modulators of GABA_A. It is believed that under the influence of hyperammonemia, both have their synthesis increased, but it is difficult to understand what emerges from the elevation of these two classes of hormones, which have antagonistic actions [26]. However, the current body of evidence supports the exploration of GABA_A receptors as potential treatment targets (e.g., pregnenolone sulfate and bicuculline) in chronic hepatic encephalopathy [29].

On the other hand, some of the effects of GABA are terminated by the action of the GABA transporter (GAT), which acts reuptaking it at the presynaptic neuron terminal [37]. Although there is disagreement over the exact location of the four subtypes of GABA transporters (GAT1–4) in pre- and postsynaptic neurons and glial cells, it is clear that a key transporter in hepatic encephalopathy is GAT3 [38]. It is found on the surface of astrocytes and microglial cells, and its action can be reversed both in the presence of chronic hyperammonemia and/or glutamatergic hyperactivity, increasing the availability of GABA in the synaptic cleft and, consequently, the GABAergic tone [28].

10. Effects on the dopaminergic system

The main dopaminergic projections originate predominantly in the neurotransmission centers of the brainstem, especially the ventral tegmental area and substantia nigra. They are modulated by glutamatergic and GABAergic neurons and, among other functions, regulate movements, reward, and cognition [37]. Three dopaminergic circuits are particularly important in the pathophysiology of chronic hepatic encephalopathy: (1) the mesocortical pathway, (2) the striatal-thalamic-cortical pathway, and (3) the nigrostriatal pathway.

The mesocortical pathway (**Figure 3**) originates in the cellular bodies of the ventral tegmental area and extends to the prefrontal cortex, where it regulates executive functions [37]. The latter correspond to a set of abilities that, in an integrated way, allow the individual to direct behaviors to goals, to evaluate the efficiency and

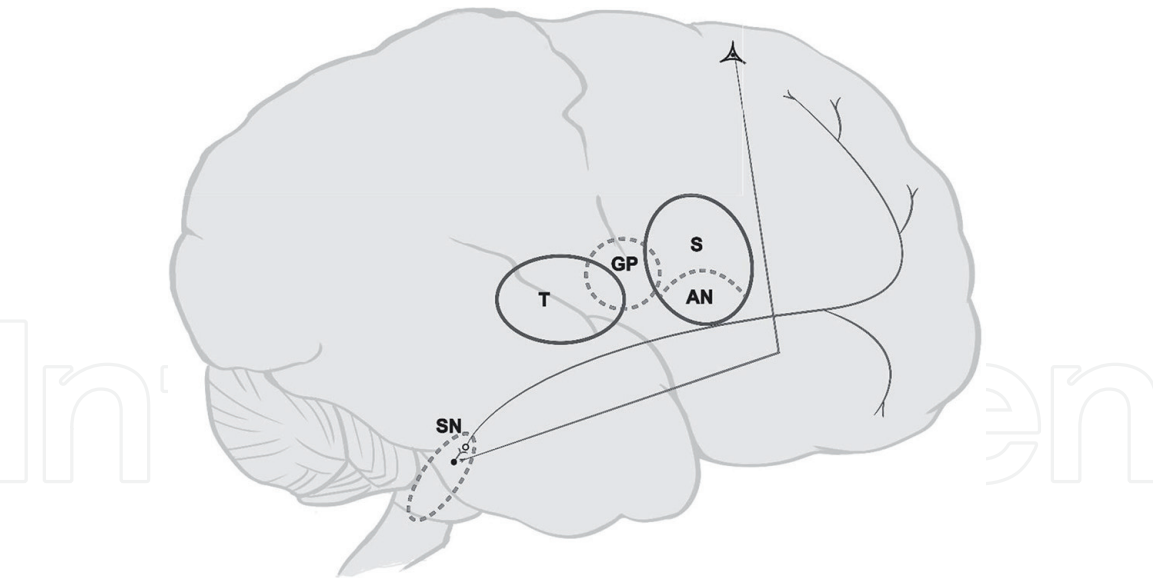


Figure 3.
The mesocortical pathway originates in the dopaminergic neurons in the ventral tegmental area and extends to the prefrontal cortex, where it regulates the executive functions. It is influenced by the activity of the frontal glutamatergic cells through GABAergic inhibitory interneurons. AN: accumbens nucleus, GP: globus pallidus, S: striatum, SN: substantia nigra, and T: thalamus.

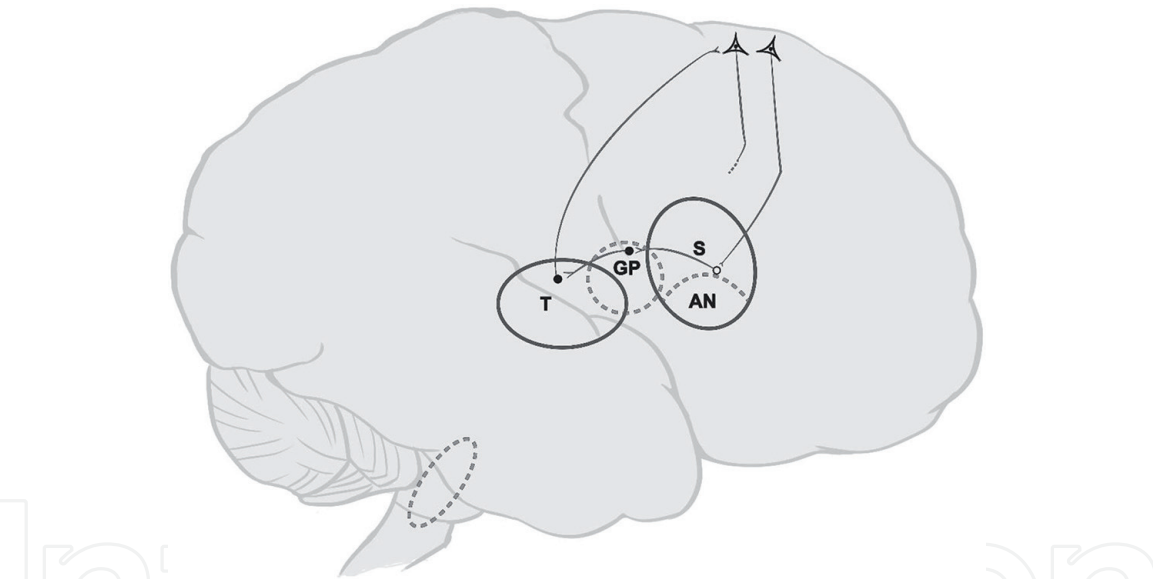


Figure 4.
The striatal-thalamic-cortical pathway originates in the dopaminergic neurons in the accumbens nucleus (AN) and extends to the globus pallidus (GP), where it regulates GABAergic interneurons that inhibit the activity of GABAergic interneurons in the thalamus (T), disinhibiting frontal glutamatergic activity. S: striatum.

adequacy of these behaviors; to abandon ineffective strategies in favor of others more efficient; and, thus, solve immediate, medium, and long-term problems [3]. It is hypothesized that the dopaminergic neurons of the ventral tegmental area are influenced by the glutamatergic neurons of the frontal descending pathway [37]. Moreover, in chronic hepatic encephalopathy, there is an increase in the activity of the enzyme monoamine oxidase B (MAO-B), with increased dopamine degradation, contributing to the development of a dysexecutive syndrome [33].

Experimental research demonstrates that the striatal-thalamic-cortical pathway (**Figure 4**) originates in the nucleus accumbens and projects to the internal globus pallidus, having an important role in the regulation of motor activity. It is believed that it is also influenced by the glutamatergic neurons from the frontal lobes, which

would excite inhibitory GABAergic interneurons. This would lead, in physiological circumstances, to a decrease in dopaminergic activity that extends from the accumbens nucleus to the internal globus pallidus, disinhibiting GABAergic interneurons that extend from the internal globus pallidus to the thalamus, where another group of GABAergic interneurons is located, with inhibitory projections to cortical glutamatergic cells. If the circuit is normofunctioning, very little dopamine is released from the accumbens nucleus, increasing the inhibitory activity that the internal globus pallidus exerts on the thalamus and preventing the latter from restricting the release of glutamate by the cortical neurons. The result is an increase in frontal glutamatergic activity, responsible for motor function. In rats submitted to a portosystemic shunt, it is observed that hyperammonemia causes greater activation of glutamatergic metabotropic receptors in the accumbens nucleus, from which results a greater release of glutamate in the frontal region, a mechanism involved in the appearance of mini-asterixis [26]. It is also hypothesized that the portosystemic shunt can promote a cerebral deposition of manganese, which characteristically generates a hypersignal in the globus pallidus in T1-weighted sequence on MRI [9]. Moreover, manganese also has a predilection for deposition in substantia nigra, with a profound toxic action on the dopaminergic neurons, which could induce or aggravate the parkinsonian symptoms of hepatic encephalopathy [10]. Human studies, however, do not demonstrate a correlation between the hyperintensity of the globus pallidus and the severity of motor symptoms [27].

The nigrostriatal pathway (**Figure 5**) extends from the dopaminergic cell bodies of the substantia nigra to the striatum, forming part of the extrapyramidal system. It is modulated by the glutamatergic pathway and the accumbens nucleus, both being connected to it through inhibitory GABAergic interneurons. In rats submitted to a portosystemic shunt, hyperammonemia causes activation of glutamatergic ionotropic AMPA receptors in the accumbens nucleus [26], and neuroinflammation decreases the expression of glutamatergic transporters EEAT1 and VGLUT1, increasing the availability of glutamate in substantia nigra [11]. The result of this glutamatergic hyperactivity is an increase in inhibition of the nigrostriatal pathway [26], whose deficiency in dopaminergic release leads to the onset of parkinsonian symptoms such as stiffness, bradykinesia, and tremor [37]. Interestingly, experimental studies show

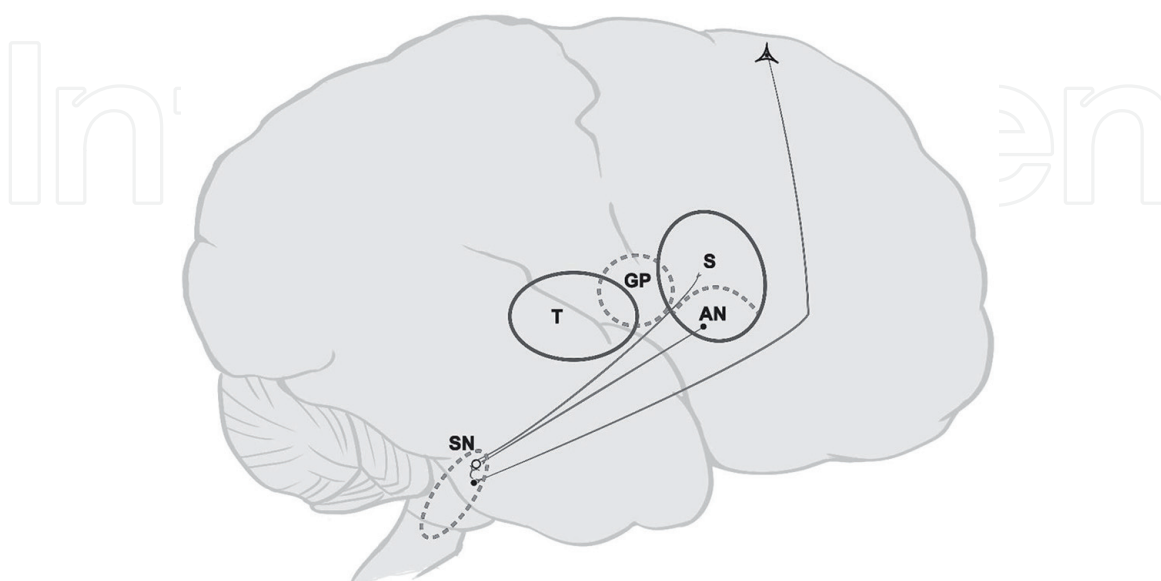


Figure 5.
 The nigrostriatal dopaminergic pathway originates from the substantia nigra (SN) and extends to the striatum (S), where it regulates the extrapyramidal system. It is inhibited by GABAergic interneurons in the accumbens nucleus (AN) or brainstem, the latter being modulated by frontal glutamatergic cells. GP: globus pallidus and T: thalamus.

that the activation of glutamatergic metabotropic receptors in the substantia nigra can also cause a decrease in the locomotion of rodents, since the substantia nigra has a second pathway of GABAergic neurons that extends into the thalamus, where a group of GABAergic interneurons inhibit motor cells, resulting in hypokinesia [26].

11. Effects on the cholinergic system

Acetylcholine is a neurotransmitter and modulator that, when bound to nicotinic receptors, favors neuronal excitability, and when bound to muscarinic receptors (mainly of the M2 subtype), inhibits the inhibitory activity triggered by the activation of GABA_A receptors, i.e., disinhibits the postsynaptic terminal [31]. Two cholinergic pathways are particularly important in the pathophysiology of hepatic encephalopathy (**Figure 6**): (1) those originating from the ascending activating reticular system in the brainstem (particularly the laterodorsal tegmental nuclei and pedunculopontine nuclei) and (2) those originating from the basal forebrain, an area that includes the nucleus basalis of Meynert, the medial septal nucleus, and the diagonal band of Broca [37].

The projections of acetylcholine that originate in the ascending reticular activating system extend to the prefrontal cortex, basal forebrain, thalamus, hypothalamus, amygdala, and hippocampus; they are considered to be involved in vigilance (sustained attention) [39]. Cholinergic neurons that originate in the basal forebrain extend to the prefrontal cortex, hippocampus, and amygdala; they are involved with the formation of episodic memory [37].

The effects of acetylcholine are terminated by two enzymes, acetylcholinesterase and butyrylcholinesterase. Both convert acetylcholine to choline, which is then transported back to the presynaptic terminal for further synthesis of this neurotransmitter [37]. Cirrhosis is associated with an increase of approximately 30% in acetylcholinesterase activity in humans, which contributes to a decrease in acetylcholine levels and a consequent potentiation of the effects of GABAergic tonus [34]. Little is known about how chronic hyperammonemia and neuroinflammation induce changes in the cholinergic system [31]. There is no correlation, for example, between serum ammonia levels and acetylcholinesterase activity [34]. However, experimental studies have shown that the increased availability of acetylcholine in

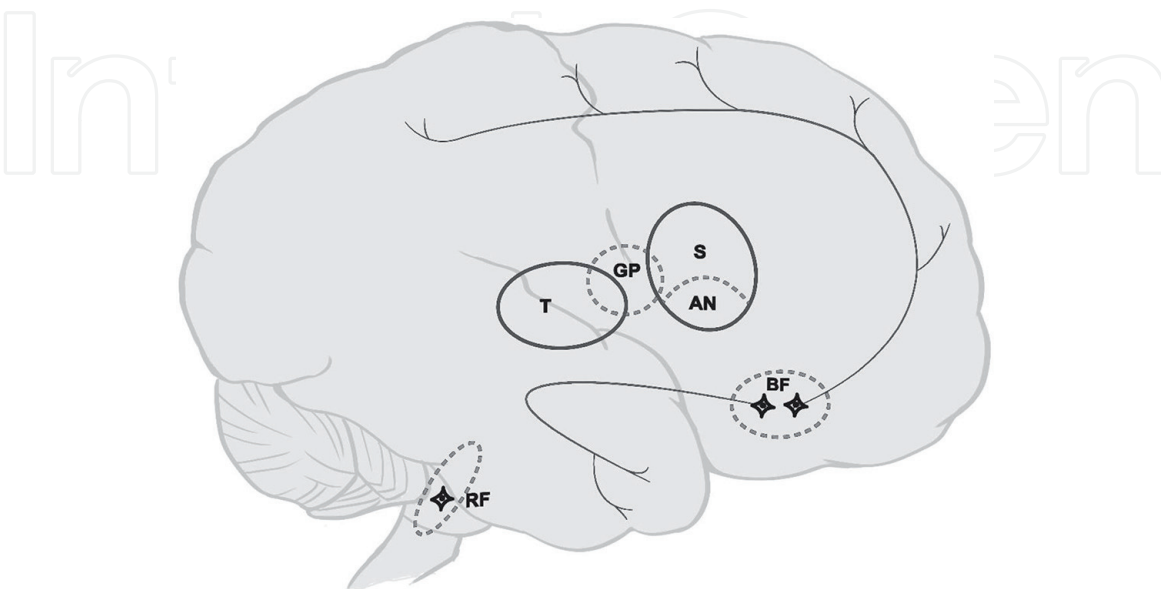


Figure 6.
The cholinergic pathways originate in the basal forebrain (BF) and reticular formation (RF). They extend to the prefrontal cortex and medial portion of the temporal cortex. AN: accumbens nucleus, GP: globus pallidus, S: striatum, and T: thalamus.

the synaptic cleft, either by direct administration or by inhibition of its degradation, is related to the reduction in glutamate neurotoxicity and improvement in the severity of hepatic encephalopathy [31].

12. Effects on the serotonergic system

Serotonin is a neurotransmitter and modulator that favors the excitability of cortical neurons; a decrease in serotonergic tone potentiates the effects of increased GABAergic tone [31]. Serotonergic neurons have both ascending and descending projections (**Figure 7**). The ascending projections originate in the raphe nuclei in the brainstem and extend to the cerebellum, hypothalamus, thalamus, amygdala, hippocampus, striatum, accumbens nucleus, basal forebrain, and prefrontal cortex [37]. They are related to the regulation of mood, hunger, impulsivity, and circadian rhythm [35]. The descending projections extend to the lower portions of the brainstem and spinal cord, being important for pain regulation [37].

The dysfunction of the serotonergic system has been widely documented in both minimal hepatic encephalopathy and overt hepatic encephalopathy: it underlies several early neuropsychiatric disorders in the disease, such as mood and sleep disorders. Serotonin levels correlate with the severity of cirrhosis and the degree of portosystemic shunt [35]. There is an increase in the circulation of l-tryptophan, the precursor amino acid of this neurotransmitter, in blood and cerebrospinal fluid. It is hypothesized that hyperammonemia not only stimulates serotonin synthesis, but also its degradation by the enzyme monoamine oxidase A (MAO-A), which is shown by the concomitant increase of the main product of its metabolism, 5-hydroxyindoleacetic acid [31, 33, 35].

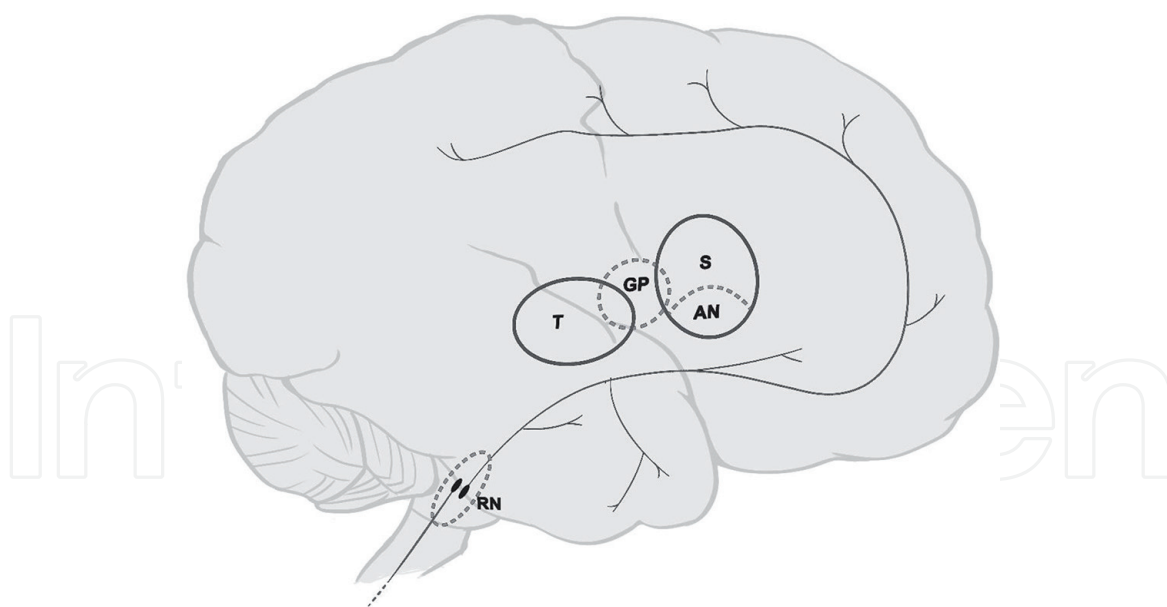


Figure 7.

The ascending serotonergic pathway originates in the raphe nucleus (RN) and extends to the medial portion of the temporal cortex and prefrontal cortex, while the descending pathway modulates the activity of the spinal cord. AN: accumbens nucleus, GP: globus pallidus, S: striatum, and T: thalamus.

13. Effects on the histaminergic system

Histamine acts in conjunction with serotonin to regulate the circadian rhythm [31]. Histaminergic neurons originate in the tuberomammillary nucleus of the hypothalamus and make extensive projection throughout the central nervous system, including the spinal cord (**Figure 8**) [37]. Significant increase in histamine

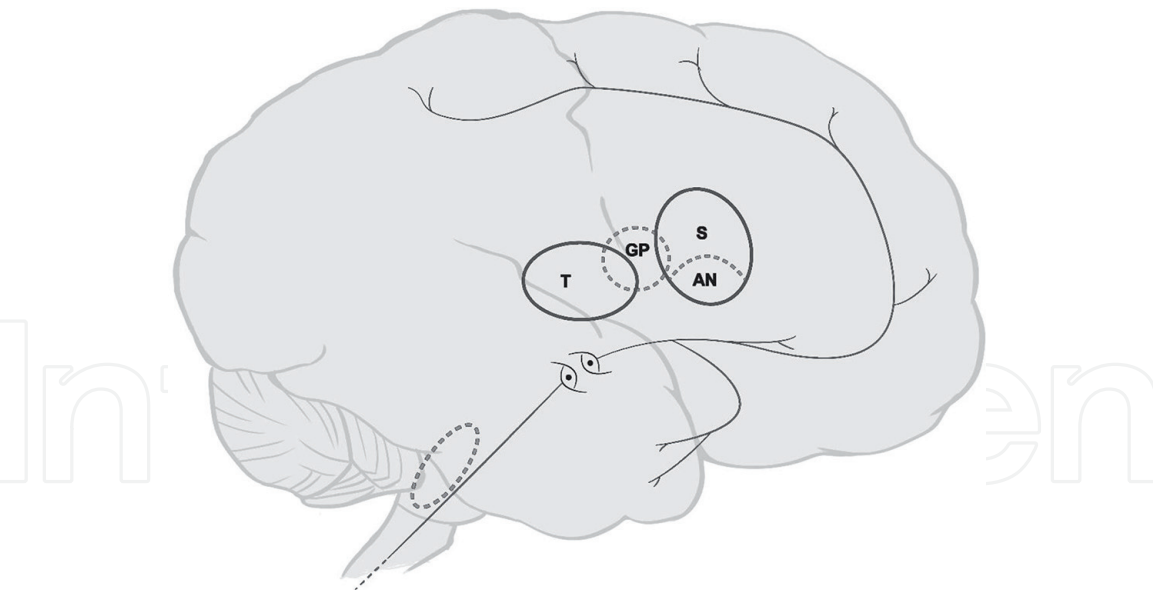


Figure 8.
The ascending histaminergic pathway originates in the hypothalamus and extends to the medial portion of the temporal cortex and prefrontal cortex, while the descending pathway modulates the activity of the spinal cord. AN: accumbens nucleus, GP: globus pallidus, S: striatum, and T: thalamus.

levels have been documented in patients with hepatic encephalopathy [31]. Histamine is produced from the amino acid histidine [37]; hyperammonemia increases both the concentration of histidine and the activity of its membrane transporter into the histaminergic neurons, stimulating the synthesis of histamine [31].

14. Effects on the noradrenergic system

In the 1970s, it was believed that hepatic encephalopathy might reflect a disturbance in catecholaminergic metabolism [31]. The main projections of noradrenaline originate in the locus coeruleus, although there are also some in the laterodorsal tegmental nuclei of the brainstem (**Figure 9**). They can be ascending or descending.

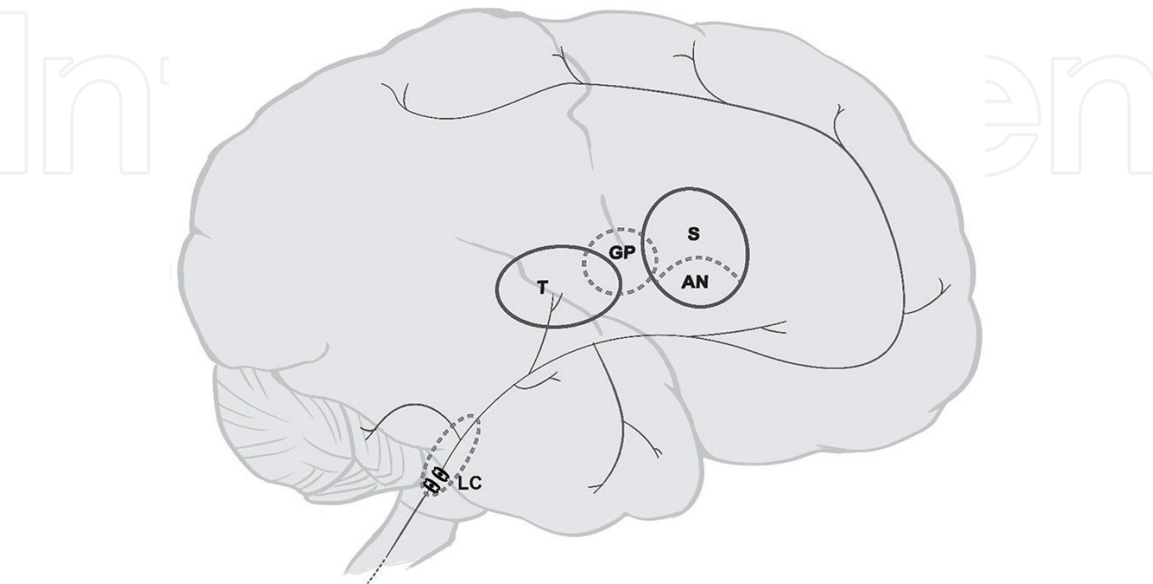


Figure 9.
The ascending noradrenergic pathway originates in the locus coeruleus (LC) and extends to the thalamus (T), medial portion of the temporal cortex, and prefrontal cortex, while the descending pathway modulates the activity of the spinal cord. AN: accumbens nucleus, GP: globus pallidus, and S: striatum.

Ascending projections regulate vigilance and mood: they end diffusely throughout the brain, including many of the same sites for which serotonergic pathways extend, although there are few noradrenergic extensions to the striatum and accumbens nucleus. The descending projections extend to the spinal cord and regulate pain [37].

Currently, it is widely accepted that changes in catecholaminergic metabolism do not precipitate hepatic coma [31]. Studies have shown that, in patients with cirrhosis, there is no decrease in norepinephrine concentration in most brain regions, with the maintenance of $\alpha 1$ and $\alpha 2$ receptor density. However, it is assumed that more subtle chronic changes may coexist with some neuropsychiatric symptoms, such as depression and anxiety [31, 33].

15. Effects on the adenosinergic system

Adenosine is a modulator of neuronal excitability, which inhibits postsynaptic potentials generated by classical neurotransmitters, such as glutamate, GABA, dopamine, and serotonin. Since 1960s, studies have shown reduced expression of adenosinergic receptors in the striatum and cortex of patients with mild hepatic encephalopathy [31].

Although the mechanisms through which adenosine exerts its function are still not fully understood [36], it is known that the decrease in the expression of its receptors occurs in the early stages of the disease and contributes to an increase in glutamatergic activity, potentializing its excitotoxic effects, while increasing GABAergic tone, also potentializing its inhibitory effects [31].

16. Final Considerations

The twentieth century provided the greatest scope of information on the neurobiology of hepatic encephalopathy throughout history, but failed to create an integrated theory that would allow the adoption of more effective intervention strategies. This was due to the reductionist view that a single process would have primacy over the others, with the emphasis on hyperammonemic theory being its greatest example. As other factors such as the composition of the intestinal microbiota, synergism with neuroinflammation, and the role of glutamatergic and GABAergic tonus balance were discovered, it became clear that this traditional and linear view of scientific research allows the understanding of the initial state of multiple dysfunctional systems, but is not able to predict the overall behavior of the disease. As twenty-first century progresses, it is imperative to incorporate concepts such as convergence, emergency, and complexity into research related to the theme, both in diachronic and synchronic processes, for the construction of a true dynamic and integrated vision that allows more effective therapeutic interventions, in a total hermeneutical cycle.

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