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# NATURAL COMPOUNDS AS MODULATORS OF NEUROSTEROIDOGENESIS: THERAPEUTIC PERSPECTIVES IN NEURODEGENERATIVE DISEASES

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*Dedico questo traguardo*
*a tutte le persone*
*a me care che,*
*da vicino o da lontano,*
*mi sono state accanto,*
*sostenendomi e accompagnandomi lungo questo percorso.*

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# Abstract

Neurodegenerative diseases are characterized by progressive neuronal dysfunction and loss, resulting in significant cognitive and motor impairment. Emerging evidence suggests that neurosteroid dysregulation contributes to the pathogenesis of these disorders by affecting neuronal survival, neurotransmission, synaptic plasticity, and neuroinflammatory responses. Neurosteroids such as allopregnanolone, pregnenolone, progesterone, and dehydroepiandrosterone (DHEA) play essential roles in maintaining central nervous system homeostasis, and alterations in their levels have been reported in several neurodegenerative diseases. This thesis reviews the role of neurosteroids in neurodegenerative disorders and explores the potential of natural compounds as modulators of neurosteroidogenesis. The literature examined indicates that neurosteroid dysregulation is a common feature of neurodegenerative diseases and may contribute to disease progression. Furthermore, selected natural compounds, including ginsenoside Rb1, palmitoylethanolamide (PEA), and vitamin C, have demonstrated the ability to modulate neurosteroid levels or stimulate neurosteroidogenic pathways. Overall, the findings highlight neurosteroidogenesis as a promising therapeutic target and suggest that natural compounds capable of enhancing endogenous neurosteroid production may offer novel strategies for neuroprotection and the management of neurodegenerative diseases. Further clinical and experimental studies are required to establish their therapeutic efficacy and long-term safety.



Keywords: Neurosteroids, Neurosteroidogenesis, Neurodegenerative Diseases, Neuroprotection, Allopregnanolone, Natural Compounds, Palmitoylethanolamide, Ginsenoside Rb1, Vitamin C

# CHAPTER 1. Neurodegenerative Diseases: An Overview

Neurodegenerative diseases are a group of neurological disorders characterized by progressive dysfunction, degeneration, and eventual loss of neurons, particularly in the brain. The term does not refer to a single disease or a specific disease state, rather, it encompasses a diverse range of disorders that share the common features of neuronal dysfunction and progressive neurodegeneration. These pathological changes can lead to the gradual deterioration of neurological functions and impair an individual's ability to perform daily activities. Neurodegenerative diseases comprise a wide range of disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Multiple Sclerosis (MS), Amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and other forms of dementia (Dugger & Dickson, 2017).

## 1.1 Alzheimer's disease

Alzheimer's disease (AD), the most common neurodegenerative disease worldwide, is a progressive neurodegenerative disease characterized by dysfunction and eventual loss of neurons in the brain, leading to memory loss, cognitive decline, and difficulties in performing daily activities. The exact cause of AD remains unclear, although several hypotheses suggest the involvement of abnormal accumulation of misfolded proteins, particularly amyloid-β and tau. The signs and symptoms of AD include memory loss, cognitive decline, behavioral changes, and progressive loss of independence. Age is the strongest known risk factor, with the risk increasing substantially after the age of 65, although younger-onset Alzheimer's disease can also occur. Genetic factors and family history also contribute to the risk of developing AD in some individuals. Other factors, including cardiovascular health, high blood pressure, obesity, diabetes, head injuries, and certain lifestyle-related factors, have also been associated with the risk of developing AD. Although there is currently no cure for Alzheimer's disease, available treatments include medications to manage cognitive and behavioral symptoms, disease-modifying therapies such as anti-amyloid immunotherapies, and lifestyle-based strategies (Breijyeh & Karaman, 2020).

## 1.2 Parkinson's disease

Parkinson's disease (PD), the second most common neurodegenerative disease worldwide, is a progressive neurodegenerative disorder characterized primarily by the dysfunction and loss of dopaminergic neurons in the substantia nigra of the brain, leading to motor and non-motor symptoms. The exact cause of PD remains unclear, although several hypotheses suggest the involvement of abnormal accumulation of misfolded proteins, particularly α-synuclein, as well as mitochondrial dysfunction, oxidative stress, and neuroinflammation. The signs and symptoms of PD include resting tremors, bradykinesia, muscle rigidity, postural instability, and various non-motor symptoms such as sleep disturbances, depression, and cognitive impairment. Age is the strongest known risk factor, with the risk increasing substantially with advancing age, although younger-onset Parkinson's disease can also occur. Genetic factors and family history contribute to the risk of developing PD in some individuals, while other factors, including environmental exposures and lifestyle-related factors, have also been associated with disease risk. Although there is currently no cure for Parkinson's disease, available treatments include medications to manage motor and non-motor symptoms, surgical approaches such as deep brain stimulation, and supportive strategies including physiotherapy and exercise (Balestrino & Schapira, 2020).

## 1.3 Multiple sclerosis

Multiple sclerosis (MS), a chronic inflammatory and neurodegenerative disease of the central nervous system, is characterized by immune-mediated damage to myelin, axons, and neurons, leading to a wide range of neurological symptoms. The exact cause of MS remains unclear, although several hypotheses suggest the involvement of an abnormal immune response against components of the central nervous system, together with genetic and environmental factors. The signs and symptoms of MS include visual disturbances, muscle weakness, numbness or tingling, impaired coordination and balance, fatigue, and cognitive difficulties. Age is an important risk factor, with MS most diagnosed between the ages of 20 and 40, although it can occur at any age. Genetic factors and family history contribute to the risk of developing MS in some individuals, while other factors, including smoking, vitamin D deficiency, certain viral infections, and environmental factors, have also been associated with disease risk. Although there is currently no cure for MS, available treatments include disease-modifying therapies to reduce disease activity and progression, medications to manage symptoms, and rehabilitation strategies such as physiotherapy and occupational therapy (Dobson & Giovannoni, 2019).

## 1.4 Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disease of the central nervous system, is characterized by the degeneration and eventual loss of upper and lower motor neurons, leading to progressive muscle weakness and loss of motor function. The exact cause of ALS remains unclear, although several hypotheses suggest the involvement of multiple mechanisms, including abnormal protein accumulation, oxidative stress, mitochondrial dysfunction, impaired RNA metabolism, and neuroinflammation. The signs and symptoms of ALS include muscle weakness, muscle stiffness, twitching, difficulty speaking and swallowing, impaired coordination, and progressive difficulty with movement and breathing. Age is an important risk factor, with ALS most commonly developing between the ages of 60 and 79, although it can occur at younger or older ages. Genetic factors and family history contribute to the risk of developing ALS in some individuals, with approximately 5–10% of cases considered familial, while the remaining cases are generally classified as sporadic. Other factors, including certain environmental and occupational exposures, have also been investigated in relation to

ALS risk. Although there is currently no cure for amyotrophic lateral sclerosis, available treatments include medications that may slow disease progression, medications to manage symptoms, respiratory and nutritional support, and multidisciplinary rehabilitation and supportive care (Feldman et al., 2022).

## 1.5 Huntington's disease

Huntington's disease (HD), a progressive inherited neurodegenerative disease of the central nervous system, is characterized by the degeneration and eventual loss of neurons in specific regions of the brain, particularly the striatum, leading to progressive motor, cognitive, and psychiatric disturbances. The exact mechanisms underlying the development and progression of HD are not fully understood, although the disease is caused by an inherited expansion of CAG repeats in the HTT gene, which leads to the production of a mutant huntingtin protein and contributes to neuronal dysfunction and degeneration. The signs and symptoms of HD include involuntary movements, muscle rigidity, impaired coordination, cognitive decline, difficulties with speech and swallowing, and behavioral and psychiatric changes. Age is an important factor in the development of HD, with symptoms most commonly appearing between the ages of 30 and 50, although juvenile and late-onset forms can also occur. Genetic factors and family history play a central role in HD, as the disease is inherited in an autosomal dominant manner, meaning that an affected individual has a 50% chance of passing the mutated gene to each child. Other factors may influence the age of onset and progression of the disease, although the CAG repeat expansion is the primary genetic determinant. Although there is currently no cure for Huntington's disease, available treatments include medications to manage motor and psychiatric symptoms, supportive therapies, and multidisciplinary approaches aimed at maintaining functional independence and quality of life (Bates et al., 2015).

The main pathological features and clinical manifestations of AD, PD, MS, ALS, and HD are summarized in Figure 1.

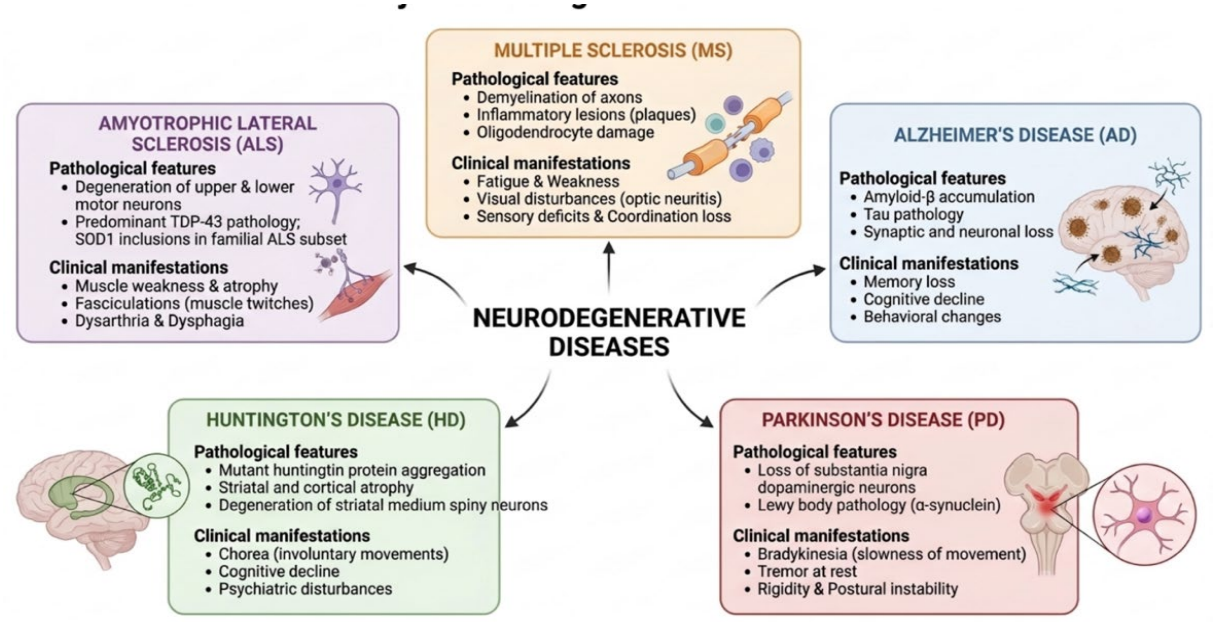



**Figure 1**. Overview of the major neurodegenerative diseases, their pathological features, and clinical manifestations. Created with Figure Lab.

According to the World Health Organization, approximately 57 million people were living with dementia worldwide in 2021. Alzheimer's disease is the most common form of dementia and is estimated to account for 60–70% of dementia cases, corresponding to approximately 34–40 million people worldwide (World Health Organization, 2026). Similarly, Parkinson's disease represents a substantial and growing global health burden. In 2021, an estimated 11.77 million people were living with Parkinson's disease worldwide, corresponding to an age-standardized prevalence rate of 138.63 per 100,000 population (95% UI: 123.06–157.62). The global age-standardized prevalence increased substantially from 86.28 per 100,000 in 1990 to 138.63 per 100,000 in 2021 (Li et al., 2025).

Multiple sclerosis also affects a considerable number of people worldwide. In 2021, approximately 1.89 million people were living with multiple sclerosis, corresponding to a global prevalence of 23.9 cases per 100,000 population (95% UI: 21.4–26.8) (Khan & Hashim, 2025) .In comparison, amyotrophic lateral sclerosis (ALS) is a rarer neurodegenerative disease, and its reported prevalence varies considerably between countries. A systematic review of 140 population-based studies reported point prevalence estimates ranging from 1.57 to 11.80 cases per 100,000 population. This variation reflects differences in disease ascertainment, availability of population-based registries, and the quality of epidemiological data across countries (Wolfson et al., 2023). Huntington's disease is also relatively rare. A systematic review and

meta-analysis of 33 epidemiological studies published between 2010 and 2022 estimated the pooled global prevalence of Huntington's disease at 4.88 cases per 100,000 persons (95% CI: 3.38–7.06) (Medina et al., 2022). Overall, these estimates demonstrate substantial differences in the global prevalence of major neurodegenerative diseases, with Alzheimer's disease and Parkinson's disease affecting considerably larger populations than multiple sclerosis, Huntington's disease, and ALS.

Beyond their high prevalence, neurodegenerative and other neurological disorders impose a substantial burden on patients, their families, healthcare systems, and economies worldwide. These conditions can lead to long-term disability, reduced quality of life, and increased demands for treatment, rehabilitation, and support services. Neurological disorders are also major contributors to mortality and disability-adjusted life years (DALYs), particularly in low- and middle-income countries, highlighting their broader social and economic impact. Reducing this burden requires effective prevention, treatment, rehabilitation, and support strategies at both national and international levels. Given the progressive nature of many neurodegenerative diseases and the limited availability of effective disease-modifying treatments, there is an urgent need for continued research to develop more effective therapeutic approaches and improve the quality of life of affected individuals and their families (Kumar & Mirza, 2025).

# CHAPTER 2. Neurosteroids and Neurosteroidogenesis: A Comprehensive Overview

Following the overview of major neurodegenerative diseases and their underlying pathophysiological mechanisms, understanding the endogenous protective and regulatory systems of the central nervous system becomes essential. Neurosteroids represent a unique class of locally synthesized steroid molecules that exert profound modulatory effects on neuronal function, synaptic plasticity, and cellular survival. This chapter provides a comprehensive overview of neurosteroid biology, beginning with their definition, historical background, and structural classification. It further examines the enzymatic pathways governing neurosteroidogenesis and explores the principal molecular targets and biological actions through which neurosteroids regulate central nervous system homeostasis.

## 2.1 Definition and Historical Perspective of Neurosteroids

Neurosteroids are endogenous steroid molecules synthesized within the nervous system that exert biological effects on neurons and glial cells. The term combines “neuro” referring to the nervous system, and “steroid” reflecting their characteristic steroidal structure. Unlike many classical steroid hormones that are primarily produced by peripheral endocrine glands and subsequently reach the brain through the circulation, neurosteroids can be synthesized locally within the central and peripheral nervous systems. Through their actions on diverse cellular targets, neurosteroids regulate numerous physiological processes, including neurotransmission, synaptic plasticity, cognition, emotional behavior, stress responses, and neuroprotection (Reddy, 2022).

Like all steroid compounds, neurosteroids share a common cyclopentanoperhydrophenanthrene nucleus consisting of three six-membered rings (A, B, and C) and one five-membered ring (D). Structural diversity arises from variations in functional groups, oxidation states, side-chain composition, and stereochemistry, resulting in a wide range of biologically active steroid molecules with distinct pharmacological properties (Hindmarsh & Geertsma, 2017). These structural modifications critically influence receptor interactions, metabolic pathways, and biological activity. As illustrated in Figure 2, variations in functional groups, oxidation states, side-chain composition, and stereochemistry of the cyclopentanoperhydrophenanthrene nucleus

generate structurally diverse neurosteroids with distinct biological activities and pharmacological properties.

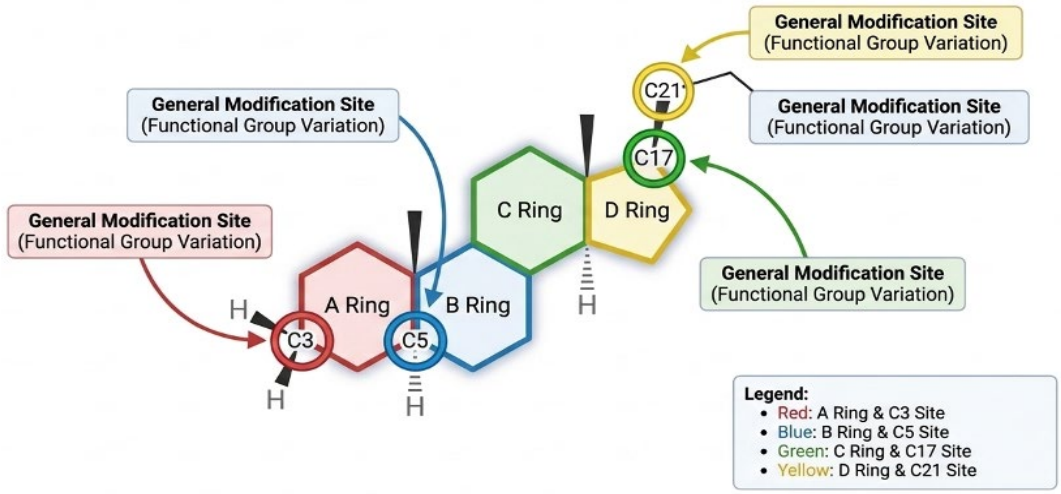



**Figure 2.** Cyclopentanoperhydrophenanthrene nucleus and key structural modification sites determining neurosteroid diversity. Created with Figure Lab.

The concept of neurosteroids emerged during the late twentieth century through observations that certain steroids and their metabolites were present in the brain at concentrations that could not be solely explained by peripheral production. A breakthrough came from the pioneering work of Baulieu, who demonstrated that the nervous system possesses the enzymatic machinery necessary for the de novo synthesis and metabolism of steroid compounds. These findings established the brain not merely as a target of circulating steroid hormones but also as an active steroidogenic organ capable of producing biologically active steroids locally (BAULIEU, 1981; Mellon & Griffin, 2002). Consequently, the term “neurosteroid” was introduced to describe steroids synthesized within the nervous system independently of peripheral endocrine sources.

A distinction should be made between neurosteroids and neuroactive steroids, two terms that are often used interchangeably but describe different concepts. Neurosteroids refer specifically to steroids synthesized within the nervous system, whereas neuroactive steroids encompass both locally synthesized steroids and circulating steroid hormones or their metabolites that can influence neuronal function. Thus, all neurosteroids are neuroactive steroids, but not all neuroactive steroids are neurosteroids.

Neurosteroids are primarily derived from cholesterol through a series of enzymatic reactions involving steroidogenic enzymes expressed in neurons and glial cells. These biosynthetic pathways generate several neuroactive compounds, including pregnenolone, dehydroepiandrosterone (DHEA), allopregnanolone, and tetrahydrodeoxycorticosterone (THDOC). Many of these molecules undergo further metabolic transformations that alter their receptor affinity and biological activity, thereby expanding the diversity of neurosteroid signaling within the nervous system (Mellon & Griffin, 2002) . Figure 3 provides a schematic overview of neurosteroid biosynthesis within the central and peripheral nervous systems, their cellular targets, and the principal biological functions mediated through genomic and non-genomic mechanisms.

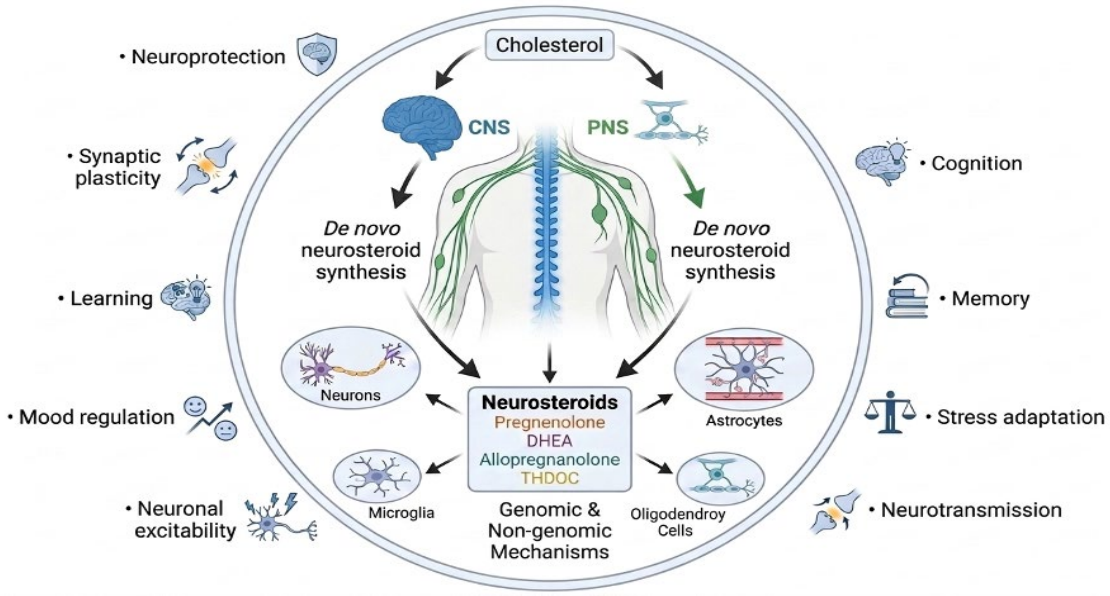



**Figure 3.** Overview of de novo neurosteroid synthesis, cellular targets, and biological functions in the nervous system. Created with Figure Lab.

The biological actions of neurosteroids are mediated through both genomic and non-genomic mechanisms. Classical genomic effects involve interactions with intracellular steroid receptors that regulate gene transcription and protein synthesis. In contrast, rapid non-genomic actions occur through direct modulation of membrane-associated neurotransmitter receptors and ion channels. Among these targets, γ-aminobutyric acid type A (GABA-A) receptors represent the most extensively studied, with several neurosteroids acting as potent positive allosteric modulators of inhibitory neurotransmission. Certain neurosteroids can also influence excitatory

signalling through interactions with N-methyl-D-aspartate (NMDA) receptors and other neuronal targets, thereby contributing to the regulation of neuronal excitability and network activity (Mellon & Griffin, 2002; Reddy, 2022).

Owing to their capacity to modulate neuronal communication, synaptic function, and cellular survival pathways, neurosteroids are now recognized as key regulators of nervous system physiology. Their involvement in stress adaptation, learning and memory, mood regulation, and neuroprotection has stimulated considerable interest in their potential therapeutic applications. Consequently, alterations in neurosteroid biosynthesis and signaling have been increasingly investigated in the context of neurological, neuropsychiatric, and neurodegenerative disorders, highlighting their significance as both biomarkers and prospective therapeutic targets.

## 2.2 Classification of Neurosteroids

To date, there is no universally accepted list encompassing all neurosteroids, nor is there a universally established classification based on their functional effects (Fedotcheva & Shimanovsky, 2025). Based on their chemical structure, neurosteroids can broadly be grouped into three major structural categories: pregnane, androstane, and sulfated neurosteroids. These broad structural categories are distinguished primarily by their steroid backbone and/or chemical modifications. Pregnane neurosteroids are characterized by a C21 steroid backbone, whereas androstane neurosteroids possess a C19 steroid backbone. Sulfated neurosteroids, in contrast, are characterized by the addition of a sulfate group to a steroid scaffold. Representative examples of each category are summarized in Table 1. Pregnane neurosteroids include allopregnanolone (ALLO), pregnenolone (PREG), progesterone (PROG), dihydroprogesterone (DHP), and tetrahydrodeoxycorticosterone (THDOC). Androstane neurosteroids include androstanediol, dehydroepiandrosterone (DHEA), and etiocholanolone, whereas dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PREGS) are examples of sulfated Neurosteroids (Reddy, 2022; Walton & Maguire, 2025). It is worth noting that neurosteroids have also been classified according to their functional effects. In this context, some authors have broadly categorized neurosteroids according to their predominant effects on target receptors as inhibitory or excitatory (Walton & Maguire, 2025) . However, this functional distinction should not be considered absolute, as the effects of individual neurosteroids may

vary depending on their molecular structure, receptor subtype, concentration, and cellular context.

| Category | Key structural feature | Representative examples |
|---|---|---|
| Pregnane | C21 steroid backbone | Allopregnanolone (ALLO)<br>Pregnenolone (PREG)<br>Progesterone (PROG)<br>Dihydroprogesterone (DHP)<br>Tetrahydrodeoxycorticosterone (THDOC) |
| Androstane | C19 steroid backbone | Androstanediol<br>Dehydroepiandrosterone (DHEA)<br>Etiocholanolone |
| Sulfated | Addition of a sulphate group to a steroid scaffold | Dehydroepiandrosterone sulfate (DHEAS)<br>Pregnenolone sulfate (PREGS) |

**Table 1.** Broad structural classification of neurosteroids

## 2.3 Neurosteroidogenesis

Neurosteroidogenesis refers to the process by which neurosteroids are synthesized within the nervous system through a series of enzymatic reactions. It primarily begins with cholesterol, which serves as the main precursor for neurosteroid production. Cholesterol can be obtained from extracellular sources or synthesized within neural cells and is transported into mitochondria, where neurosteroidogenesis is initiated. The first major step involves the conversion of cholesterol into pregnenolone by the mitochondrial enzyme cytochrome P450 side-chain cleavage enzyme (CYP11A1, P450scc). Pregnenolone serves as a key intermediate and can subsequently be converted into several steroids, including progesterone and dehydroepiandrosterone (DHEA). These intermediates undergo further enzymatic transformations involving enzymes such as 3β-hydroxysteroid dehydrogenase (3β-HSD), CYP17A1, 5α-reductase, and 3α-hydroxysteroid dehydrogenase (3α-HSD), ultimately generating a variety of neurosteroids. For example, progesterone can be converted by 5α-reductase into 5α-reduced metabolites, which can subsequently be converted by 3α-HSD into neuroactive steroids such as allopregnanolone. The profile of neurosteroids produced varies among different neural cells and brain regions according to the expression and activity of

steroidogenic enzymes. Neurosteroidogenesis is tightly regulated by factors including cholesterol availability, mitochondrial cholesterol transport, steroidogenic enzyme expression and activity, and intracellular signaling mechanisms. In addition, physiological and pathological conditions, including stress, hormonal changes, neuronal activity, and aging, can influence the synthesis and availability of neurosteroids. Through these regulated pathways, the nervous system produces neurosteroids that can modulate neuronal activity and brain function, particularly through interactions with neurotransmitter receptors such as GABA-A receptors (Do Rego et al., 2009; Porcu et al., 2016). The major pathways involved in neurosteroidogenesis are presented in Figure 4, reproduced from (Cai et al., 2018).

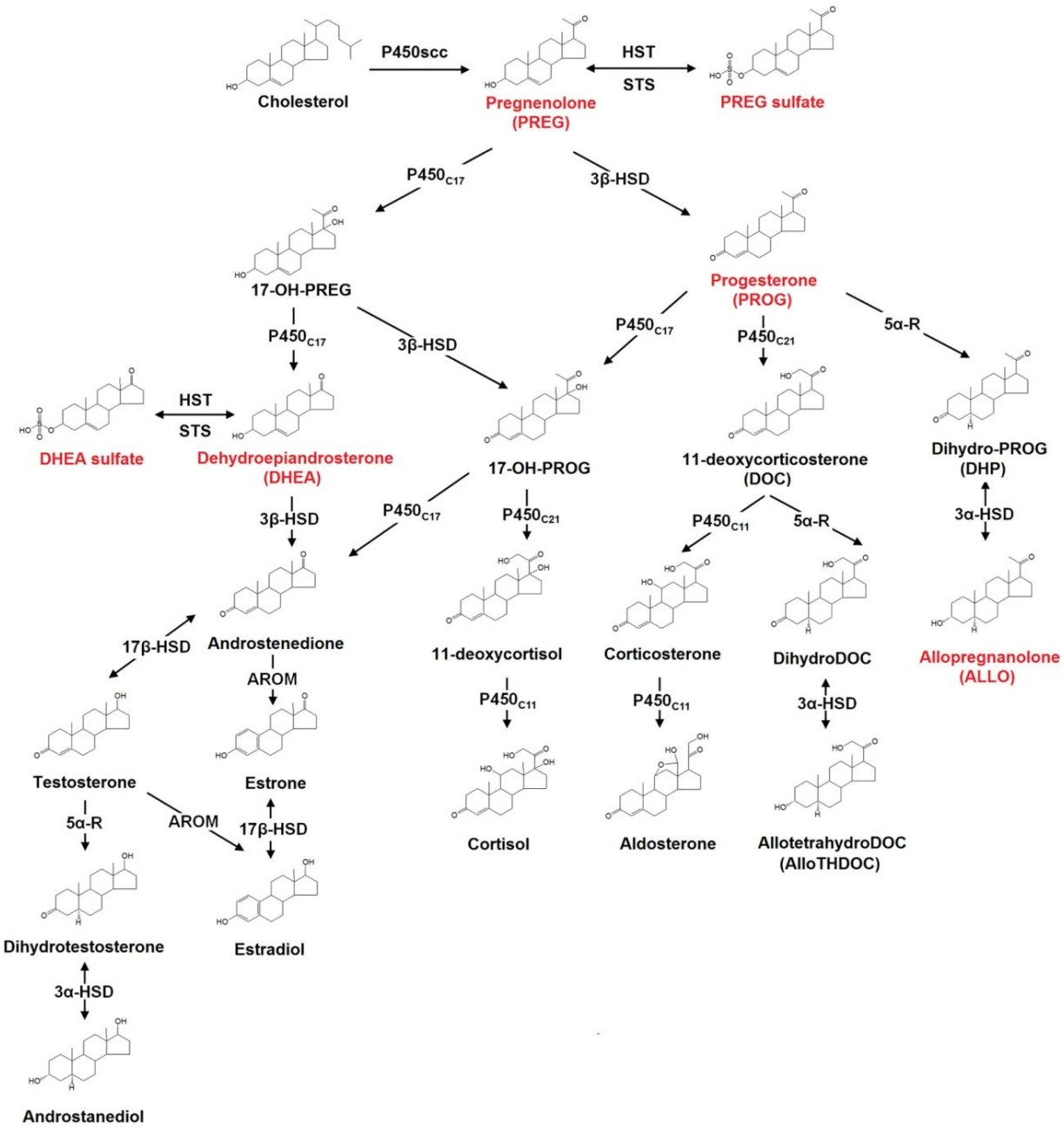



**Figure 4.** Major enzymatic pathways of neurosteroid biosynthesis from cholesterol in the human brain. (Cai et al., 2018)

## 2.4 Major Targets and Biological Actions of Neurosteroids in the Central Nervous System

The biological actions of neurosteroids are diverse and closely related to the structural diversity and complex metabolism of these compounds. As discussed previously in Section 2.2, neurosteroids comprise a heterogeneous group of steroid molecules for which a comprehensive

classification is not always straightforward. Moreover, as illustrated in Section 2.3, steroid metabolism involves multiple enzymatic pathways that generate a wide range of steroid derivatives. Consequently, neurosteroids can exert a broad spectrum of biological effects, depending on their chemical structure, site of synthesis, metabolic pathways, and molecular targets. Among the major targets of neurosteroids in the central nervous system (CNS) are (GABA-A) receptors, which mediate the principal form of fast inhibitory neurotransmission in the brain. Depending on their chemical structure and concentration, neurosteroids can either positively or negatively modulate GABA-A receptor activity. Neurosteroids such as allopregnanolone (ALLO) and tetrahydrodeoxycorticosterone (THDOC) generally act as positive allosteric modulators of GABA-A receptors, enhancing GABA-induced chloride influx and thereby promoting neuronal hyperpolarization and inhibition. Other neurosteroids, particularly sulfated neurosteroids such as pregnenolone sulfate (PREG-S) and dehydroepiandrosterone sulfate (DHEA-S), can reduce GABA-A receptor activity and consequently decrease neuronal inhibition. Through these actions, neurosteroids contribute to the regulation of the balance between neuronal excitation and inhibition in the CNS. Modulation of GABA-A receptor activity by neurosteroids is therefore involved in several physiological and behavioral processes, including stress responses, anxiety, sleep, seizure susceptibility, and neuronal excitability. Changes in neurosteroid levels can also alter GABAergic inhibition, which may contribute to fluctuations in neuronal excitability under conditions such as stress or hormonal changes (Olsen, 2018; Tuem & Atey, 2017). The main effects of neurosteroids on GABA-A receptor activity and their associated consequences in the CNS are summarized in Figure 5.

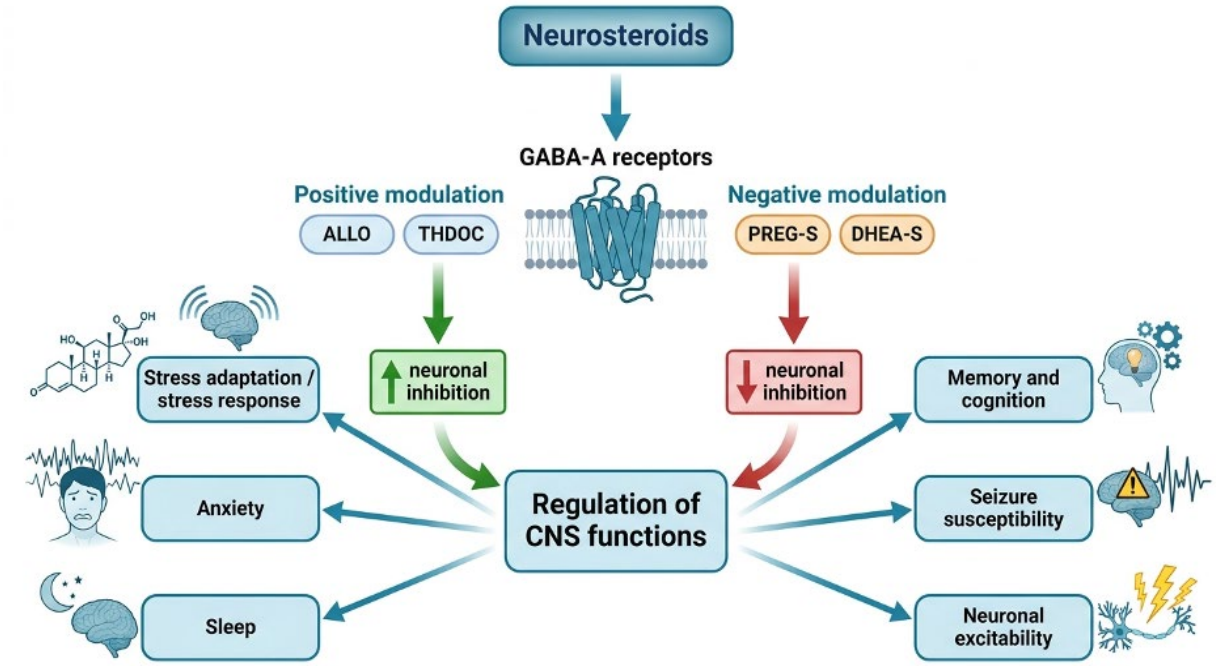



**Figure 5**. Modulation of GABA-A receptor activity by neurosteroids and its effects on central nervous system functions. Created with Figure Lab.

### 2.4.1 Modulation of NMDA Receptors

Neurosteroids can also modulate N-methyl-D-aspartate (NMDA) receptors, which are important mediators of excitatory glutamatergic neurotransmission and synaptic plasticity in the central nervous system. Pregnenolone sulfate (PREG-S) and pregnenolone hemisuccinate (PHS) have been reported to positively modulate NMDA receptor activity, enhancing glutamatergic signaling and calcium influx. Through these effects, neurosteroids may influence synaptic plasticity and long-term potentiation, processes that are fundamental to learning and memory. The modulation of NMDA receptor activity by neurosteroids therefore represents an additional mechanism through which these compounds can regulate neuronal excitability and cognitive functions in the CNS (Hansen et al., 2018; Tuem & Atey, 2017) . A schematic representation of the effects of neurosteroids on NMDA receptor activity and their associated functional consequences is shown in Figure 6.

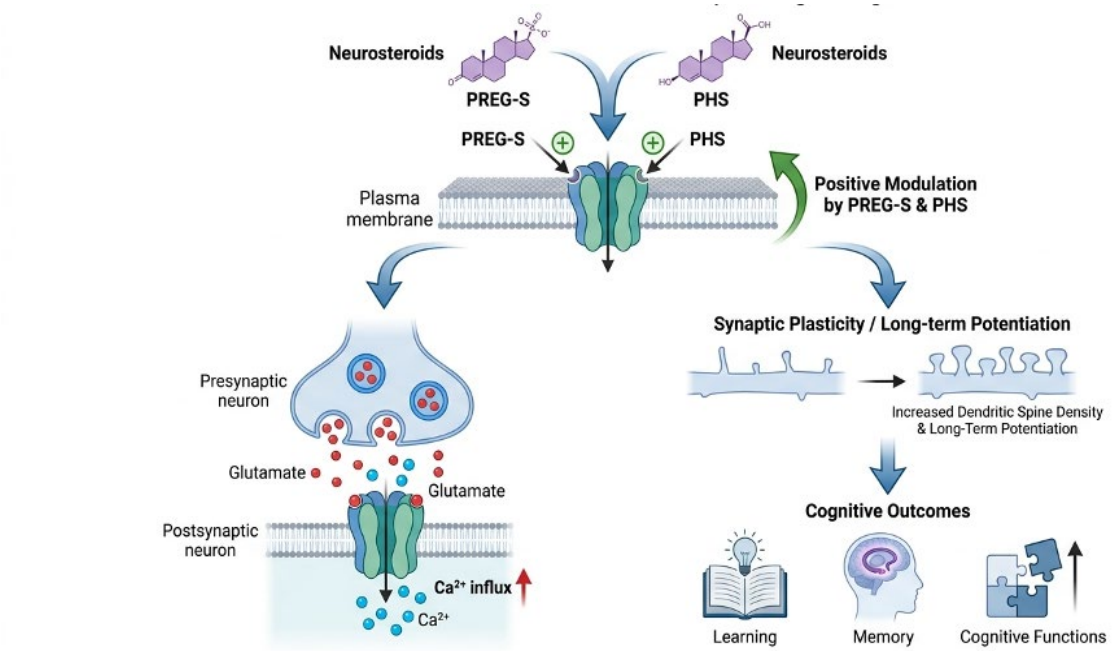



**Figure 6.** Positive modulation of NMDA receptor signaling by neurosteroids and its impact on synaptic plasticity and cognitive functions. Created with Figure Lab.

### 2.4.2 Modulation of Voltage-Gated Calcium Channels

Neurosteroids can also modulate voltage-gated calcium channels, thereby influencing intracellular calcium signaling and neuronal function in the central nervous system. Allopregnanolone has been reported to interact with L-type voltage-gated calcium channels and to influence calcium-dependent processes involved in neural progenitor cell proliferation and neurogenesis. Through the modulation of calcium influx, neurosteroids may therefore contribute to the regulation of neuronal development and plasticity. In addition, certain neurosteroids can modulate T-type voltage-gated calcium channels, although much of the evidence for these effects concerns peripheral sensory neurons. Overall, the interaction of neurosteroids with voltage-gated calcium channels represents an additional mechanism through which they can regulate calcium-dependent cellular processes and neuronal function in the CNS (Berger & Bartsch, 2014; Tuem & Atey, 2017; Weiss & Zamponi, 2019). Overall, the interaction of neurosteroids with voltage-gated calcium channels represents an additional mechanism through which they can regulate calcium-dependent cellular processes and neuronal function in the CNS. A schematic representation of the effects of neurosteroids on L-type voltage-gated calcium channels and their associated cellular consequences is shown in Figure 7.

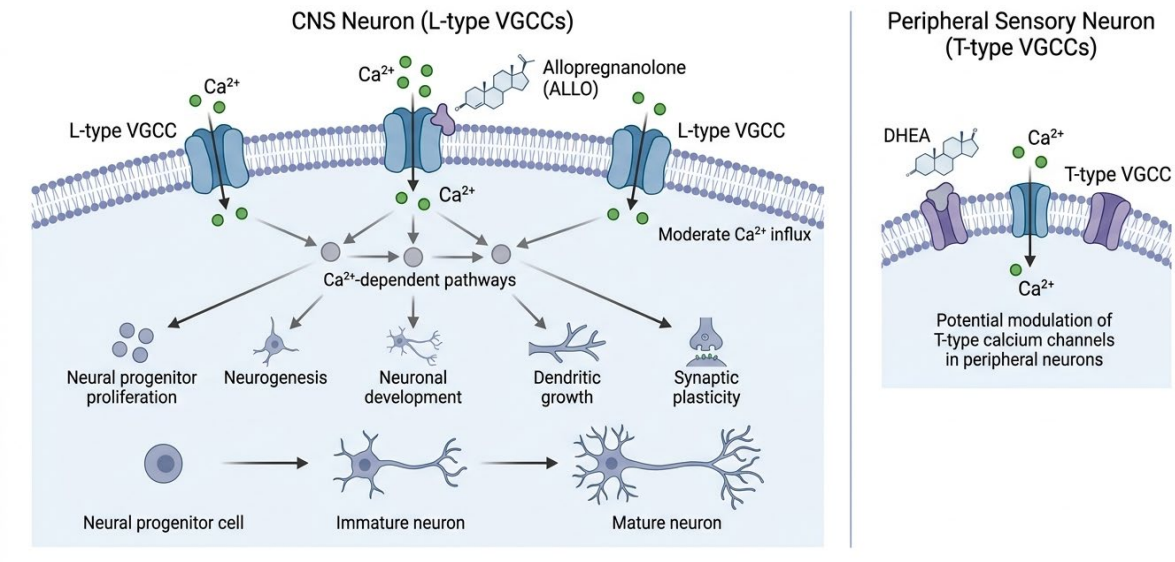



**Figure 7**. Modulation of voltage-gated calcium channels by neurosteroids in the nervous system. Created with Figure Lab.

Neurosteroids, particularly allopregnanolone (ALLO), can modulate L-type voltage-gated calcium channels (VGCCs), influencing calcium influx and activating calcium-dependent signaling pathways. These effects may contribute to neural progenitor cell proliferation, neurogenesis, neuronal development, dendritic growth, and synaptic plasticity. Certain neurosteroids, including dehydroepiandrosterone (DHEA), may also modulate T-type VGCCs, particularly in peripheral sensory neurons. ALLO, allopregnanolone; DHEA, dehydroepiandrosterone; VGCCs, voltage-gated calcium channels.

# CHAPTER 3. Neurosteroid Dysregulation in Neurodegenerative Diseases

Neurosteroids play essential roles in the maintenance of neuronal survival, synaptic plasticity, neuroinflammation, and myelin integrity. Growing evidence indicates that alterations in neurosteroid synthesis and concentrations occur in several neurodegenerative disorders, potentially contributing to disease onset and progression. Changes in the levels of neurosteroids such as allopregnanolone, pregnenolone, dehydroepiandrosterone (DHEA), and progesterone have been reported in both the central nervous system and peripheral tissues of affected individuals. These alterations may influence multiple pathological processes, including oxidative stress, neuroinflammation, mitochondrial dysfunction, protein aggregation, and neuronal loss. This chapter summarizes the currently available evidence regarding neurosteroid dysregulation in major neurodegenerative diseases and highlights disease-specific changes in neurosteroid profiles and their potential pathological significance (Brinton, 2013).

## 3.1 Alzheimer’s Disease

The first comparison of neurosteroid concentrations between patients with Alzheimer's disease (AD) and age-matched cognitively healthy controls was conducted by Weill-Engerer et al. (2002). Using high-performance liquid chromatography coupled with gas chromatography-mass spectrometry (HPLC/GC-MS), the authors quantified several neurosteroids in multiple brain regions, including the hippocampus, amygdala, frontal cortex, striatum, hypothalamus, and cerebellum. The study demonstrated significant alterations in neurosteroid profiles in AD brains, particularly reductions in dehydroepiandrosterone sulfate (DHEAS) and pregnenolone sulfate (PREGS), two neurosteroids with well-documented neuroprotective properties. Moreover, decreased levels of these neurosteroids were associated with increased concentrations of pathological AD markers, including β-amyloid (Aβ) and paired helical filament tau (PHF-tau). These findings provided some of the earliest evidence that neurosteroid dysregulation may contribute to AD pathogenesis and suggested a potential protective role for sulfated neurosteroids against neurodegenerative processes (Weill-Engerer et al., 2002).

Building on these findings, Naylor et al. (2010) further investigated neurosteroid alterations in the temporal cortex of patients with Alzheimer's disease. The study demonstrated a significant

reduction in allopregnanolone levels in AD patients compared with cognitively intact controls. Moreover, allopregnanolone concentrations were negatively correlated with Braak neuropathological stage, indicating a progressive decline with increasing disease severity. In contrast, both dehydroepiandrosterone (DHEA) and pregnenolone levels were significantly elevated in AD brains and showed positive correlations with Braak stage. The authors proposed that reduced allopregnanolone levels may contribute to AD pathogenesis because of the neurosteroid's neuroprotective properties, whereas the elevations in DHEA and pregnenolone may represent compensatory responses to ongoing neurodegenerative processes. Additionally, carriers of the APOE4 allele exhibited significantly lower allopregnanolone levels than non-carriers, suggesting a potential association between genetic risk factors and disrupted neurosteroid homeostasis in AD (Naylor et al., 2010).

Luchetti et al. (2011) comprehensively reviewed the available evidence regarding neurosteroid alterations in neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS). The authors highlighted that neurosteroids play important roles in neuronal survival, neurotransmission, glial differentiation, myelination, and neuroinflammatory regulation. Based on the available literature, they concluded that AD is characterized by significant alterations in both neurosteroid concentrations and the expression of enzymes involved in neurosteroidogenesis. The review further suggested that some of these changes may contribute to disease pathogenesis, whereas others may represent endogenous compensatory or neuroprotective responses to ongoing neurodegeneration. In particular, the authors emphasized the potential importance of neurosteroids that modulate GABAA receptor activity, such as allopregnanolone, as well as disease-associated alterations in GABAA receptor subunits that may further influence neurosteroid actions (Lucchi et al., 2021; Luchetti et al., 2011). Table 2 provides a summary of the reported changes in neurosteroid levels in AD, their associations with pathological markers and disease progression, and the potential implications of these alterations for AD pathophysiology.

| Neurosteroid | Change in AD | Association with Disease Progression | Proposed Significance | References |
|---|---|---|---|---|
| Pregnenolone sulfate (PREGS) | ↓ Decreased | Associated with increased Aβ pathology | Loss of neuroprotective effects | (Weill-Engerer et al., 2002) |
| Dehydroepiandrosterone sulfate (DHEAS) | ↓ Decreased | Associated with increased PHF-tau and Aβ pathology | Reduced neuroprotection | (Weill-Engerer et al., 2002) |
| Allopregnanolone (ALLO) | ↓ Decreased | Negatively correlated with Braak stage | May contribute to AD pathogenesis because of reduced neuroprotection and impaired GABAergic modulation | (Naylor et al., 2010) |
| Dehydroepiandrosterone (DHEA) | ↑ Increased | Positively correlated with Braak stage | Possible compensatory neuroprotective response | |
| Pregnenolone | ↑ Increased | Positively correlated with Braak stage | Possible compensatory response to neurodegeneration | (Naylor et al., 2010) |

**Table 2.** Alterations in neurosteroid levels and their association with Alzheimer's disease progression. Summary of reported changes in selected neurosteroids in Alzheimer's disease (AD), their associations with pathological and clinical measures of disease progression, proposed biological significance, and relevant references.

## 3.2 Parkinson Disease

Di Michele et al. (2003) investigated alterations in neuroactive steroid levels in patients with Parkinson's disease (PD), focusing on allopregnanolone and 5α-dihydroprogesterone (DHP). Using a gas chromatography-mass spectrometry method, the authors quantified these neuroactive steroids in the plasma of 11 PD patients and in the cerebrospinal fluid (CSF) of 12 additional patients. Compared with healthy controls, both patient groups showed significant reductions in DHP and allopregnanolone concentrations. These findings provided early evidence of altered neurosteroid homeostasis in PD and suggested that decreased levels of these neuroactive steroids could potentially serve as biochemical markers of the disease. Given the important role of GABAergic neurotransmission in the regulation of substantia nigra neurons, together with the ability of allopregnanolone and DHP to modulate GABAergic signaling, the authors proposed that the observed reductions may reflect a broader dysregulation of the GABAergic system in PD. Furthermore, because DHP has been associated with the stimulation of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), its reduction could contribute to the loss of neuroprotective mechanisms and thereby increase the vulnerability of dopaminergic neurons to degeneration (di Michele et al., 2003).

Building on the evidence of altered neuroactive steroid levels in Parkinson's disease, Luchetti et al. (2010) investigated whether these alterations were associated with changes in the neurosteroidogenic pathways within the Parkinsonian brain. The authors examined post-mortem brain tissue from patients with Parkinson's disease and neurologically healthy controls, focusing on the substantia nigra, caudate nucleus, and putamen. The expression of 37 genes involved in neurosteroid biosynthesis, steroid receptor signaling, and GABA-A receptor function was analyzed. In the substantia nigra, a significant reduction in the expression of 5α-reductase type 1 (5α-R1), an enzyme involved in the conversion of progesterone and other steroid precursors into neuroactive metabolites, was observed, together with decreased expression of sulfotransferase family 2B member 1 (SULT2B1). In contrast, alterations in neurosteroidogenic and GABAergic-related genes were also detected in the caudate nucleus, including increased expression of 3α-hydroxysteroid dehydrogenase type 3 and the GABA-A receptor α4 subunit. These findings demonstrated that Parkinson's disease is associated not only with changes in neuroactive steroid concentrations but also with alterations in the enzymes and receptors involved in their synthesis and signaling. Given the important role of neurosteroids in modulating GABAergic neurotransmission and promoting neuronal survival, the authors proposed that disruption of neurosteroid biosynthesis and GABA-A receptor signaling may contribute to the pathophysiology of Parkinson's disease, particularly within the substantia nigra, where progressive degeneration of dopaminergic neurons represents a central feature of the disease (Luchetti et al., 2010).

Further evidence supporting the involvement of neuroactive steroids in Parkinson's disease was provided by Melcangi et al. (2011), who investigated alterations in neuroactive steroid levels using an experimental model of nigrostriatal degeneration. Male rats received stereotaxic injections of the neurotoxin 6-hydroxydopamine (6-OHDA) to induce degeneration of nigrostriatal dopaminergic neurons, and neuroactive steroid concentrations were subsequently quantified in the striatum and cerebral cortex using liquid chromatography-tandem mass spectrometry. Among the wide range of steroids analyzed, including pregnenolone, progesterone, dihydroprogesterone (DHP), allopregnanolone, isopregnanolone, testosterone, dihydrotestosterone, dehydroepiandrosterone, and estrogens, significant reductions in pregnenolone and DHP were observed in the striatum. DHP levels were also significantly reduced in the cerebral cortex, whereas isopregnanolone concentrations were increased in both

the striatum and cerebral cortex. These findings demonstrated that nigrostriatal degeneration is associated with marked alterations in neuroactive steroid metabolism, particularly within the progesterone metabolic pathway. The authors suggested that such changes may reflect a disruption of neurosteroid homeostasis during dopaminergic neurodegeneration and further support a potential role for neuroactive steroids in the pathophysiology of Parkinson's disease (Melcangi et al., 2011). A summary of the reported neuroactive steroid alterations, neurosteroidogenic pathways, and experimental models investigated in Parkinson's disease is provided in Table 3.

| Study Type | Neurosteroids/pathways | Main findings | References |
|---|---|---|---|
| Human | Allopregnanolone, DHP | ↓ Allopregnanolone and DHP in plasma and CSF of PD patients | (di Michele et al., 2003) |
| Human post-mortem | 5α-R1, SULT2B1, 3α-HSD, GABA-A receptors | Altered neurosteroidogenic enzymes and GABA-A receptor subunits in the substantia nigra and basal ganglia | (Luchetti et al., 2010) |
| Animal model | Pregnenolone, DHP, allopregnanolone, isopregnanolone and others | ↓ Pregnenolone/DHP<br>↑ isopregnanolone following nigrostriatal degeneration | (Melcangi et al., 2011) |

**Table 3**. Summary of neuroactive steroid alterations and associated neurosteroidogenic pathways reported in human and experimental Parkinson's disease.

## 3.3 Multiple Sclerosis

Noorbakhsh et al. (2011) investigated alterations in neurosteroidogenesis in multiple sclerosis (MS) by examining cerebral white matter from patients with MS and non-MS controls using microRNA profiling, reverse transcription–polymerase chain reaction, immunoblotting and gas chromatography–mass spectrometry. The authors identified increased expression of three microRNAs associated with neurosteroidogenic enzymes, miR-338, miR-155 and miR-491, in MS white matter, which was accompanied by suppression of key enzymes involved in neurosteroid synthesis, including 5α-reductase type 1 and 3α-hydroxysteroid dehydrogenase. Consistent with these enzymatic alterations, levels of the neurosteroids allopregnanolone and dehydroepiandrosterone were significantly reduced in MS white matter, whereas pregnenolone levels remained unchanged. These findings were subsequently reproduced in the experimental autoimmune encephalomyelitis model, in which induction of neurosteroid-related microRNAs was associated with reduced neurosteroidogenic enzyme expression and diminished allopregnanolone levels. Importantly, treatment with allopregnanolone ameliorated

neuroinflammation, myelin and axonal injury, and neurobehavioral deficits in experimental autoimmune encephalomyelitis. Collectively, these findings provided evidence for impaired neurosteroidogenesis in MS and suggested that reduced allopregnanolone availability may contribute to disease-related neuropathology, while also highlighting the potential therapeutic and biomarker relevance of neurosteroid-based mechanisms in MS (Noorbakhsh et al., 2011).

Caruso et al. (2014) investigated alterations in neuroactive steroid levels in male patients with relapsing-remitting multiple sclerosis (RRMS) by measuring a broad panel of steroids in both cerebrospinal fluid (CSF) and plasma using liquid chromatography–tandem mass spectrometry. In the CSF of MS patients, pregnenolone and isopregnanolone levels were increased, whereas the progesterone metabolites dihydroprogesterone (DHP) and tetrahydroprogesterone (THP; allopregnanolone) were significantly decreased. Alterations were also observed in the testosterone pathway, with increased levels of 5α-androstane-3α,17β-diol and 17β-estradiol accompanied by reduced dihydrotestosterone levels. In plasma, pregnenolone, progesterone and dihydrotestosterone were increased, while DHP and THP were decreased. Thus, although changes were detected in both compartments, the pronounced alterations in progesterone and testosterone metabolites in CSF suggested a substantial disruption of neuroactive steroid metabolism within the central nervous system of male patients with RRMS. These findings further supported the involvement of neuroactive steroid pathways in MS and raised the possibility that altered steroid profiles may have diagnostic or therapeutic relevance in the disease (Caruso et al., 2014).

Orefice et al. (2016) investigated whether neuroactive steroid levels in cerebrospinal fluid (CSF) were associated with disease activity in patients with relapsing-remitting multiple sclerosis (RR-MS). The study evaluated pregnenolone (PREG), dehydroepiandrosterone (DHEA), and allopregnanolone (ALLO) in 32 RR-MS patients, including individuals experiencing an acute clinical relapse and patients in a stable phase, and compared them with patients with non-inflammatory neurological disorders and individuals without neurological disease. CSF levels of PREG and DHEA were significantly increased in both male and female RR-MS patients compared with both control groups, whereas ALLO levels were significantly lower in female RR-MS patients than in male RR-MS patients and healthy females. During clinical relapse, female RR-MS patients exhibited higher PREG levels than those observed during the stable phase, while differences in ALLO levels were also observed according to sex. Furthermore,

patients with gadolinium-enhancing lesions displayed distinct neuroactive steroid profiles, with increased PREG and DHEA in male patients and higher ALLO levels in both male and female patients compared with patients without enhancing lesions. These findings suggested that neuroactive steroid metabolism is influenced by disease activity and may differ according to sex and inflammatory lesion status. The authors therefore proposed that endogenous neuroactive steroids, particularly PREG, DHEA and ALLO, could potentially serve as biomarkers of MS activity and clinical relapse (Orefice et al., 2016).

Cheng et al. (2021) investigated whether plasma neuroactive steroid profiles were associated with disease progression during the transition from clinically isolated syndrome (CIS) to relapsing-remitting multiple sclerosis (RRMS). The study included healthy controls, patients with CIS, and patients with RRMS, with clinical disability assessed using the Expanded Disability Status Scale and matched plasma neuroactive steroids and amino acids measured. During a mean follow-up of 1.85 years, 51.6% of patients with CIS converted to RRMS. Compared with CIS patients, those with RRMS showed significantly higher plasma allopregnanolone levels and lower epiallopregnanolone levels, together with alterations in several amino acids. Importantly, within the CIS and RRMS groups, multivariate regression analyses indicated that higher plasma tetrahydrodeoxycorticosterone (THDOC) concentrations were associated with subsequent disability worsening. The authors therefore suggested that alterations in the plasma neuroactive steroid profile accompany the transition from CIS to RRMS and that THDOC, together with pregnanolone, may have potential as biomarkers of disability progression. These findings extended previous observations of altered neuroactive steroid metabolism in MS by linking specific steroid profiles to clinical disease progression (Cheng et al., 2021). Overall, these studies indicate that neuroactive steroid metabolism is altered at multiple levels in MS, involving impaired neurosteroidogenesis, changes in circulating and cerebrospinal fluid steroid profiles, and associations with disease activity and disability progression, as summarized in Table 4.

| Study | Main finding |
|---|---|
| (Noorbakhsh et al., 2011) | Impaired neurosteroidogenesis and reduced ALLO/DHEA in MS |
| (Caruso et al., 2014) | Altered neuroactive steroid profiles in CSF and plasma |
| (Orefice et al., 2016) | Neurosteroid alterations associated with disease activity |
| (Cheng et al., 2021) | Neurosteroid profile associated with MS progression/disability |

**Table 4**. Summary of studies investigating neuroactive steroid alterations and their association with disease activity and progression in multiple sclerosis.

## 3.4 Amyotrophic lateral sclerosis

Lucchi et al. (2024) investigated alterations in neurosteroid levels in patients with amyotrophic lateral sclerosis (ALS) by measuring neurosteroids in cerebrospinal fluid (CSF) from 37 ALS patients and 28 healthy controls using liquid chromatography–electrospray tandem mass spectrometry. Pregnenolone, progesterone, allopregnanolone, pregnanolone, and testosterone levels were significantly reduced in the CSF of ALS patients compared with healthy controls. Furthermore, an inverse correlation was observed between neurosteroid and neurofilament levels, suggesting a relationship between reduced neurosteroid availability and neurodegeneration. However, neurosteroid concentrations were not associated with disease progression, clinical phenotype, genotype, or survival. These findings indicate that neurosteroid dysregulation in the CSF of ALS patients may be involved in disease pathogenesis or may represent a consequence of disease-related processes. The inverse association between neurosteroid and neurofilament levels may further suggest an insufficient compensatory neuroprotective response to ongoing neurodegeneration (Lucchi et al., 2024) . The main findings regarding neurosteroid alterations in ALS are summarized in Table 5.

| Study | Main finding |
|---|---|
| Lucchi et al. (2024) | Reduced CSF levels of pregnenolone, progesterone, allopregnanolone, pregnanolone, and testosterone in ALS; neurosteroid levels inversely correlated with neurofilament levels but were not associated with disease progression or survival. |

**Table 5**. Summary of neurosteroid alterations in amyotrophic lateral sclerosis

## 3.5 Huntington's disease

In contrast to other neurodegenerative diseases, evidence regarding alterations in neurosteroid levels or dysregulation of neurosteroidogenesis in Huntington's disease (HD) remains limited. To date, there is insufficient evidence from human CNS studies to establish a consistent pattern of neurosteroid dysregulation in HD. Therefore, HD was not included in the comparative analysis of disease-associated neurosteroid alterations (Tu et al., 2026).

# CHAPTER 4. Regulation of Neurosteroidogenesis by Natural Compounds: Emerging Strategies for Neuroprotection

As discussed in the previous chapters, dysregulation of neurosteroidogenesis is increasingly recognized as an important contributor to the pathophysiology of neurodegenerative diseases. The growing understanding of neurosteroid-mediated neuroprotection has prompted considerable interest in strategies capable of restoring or enhancing endogenous neurosteroid synthesis. While direct neurosteroid replacement therapies have shown promising results in experimental and clinical studies, concerns related to pharmacokinetics, bioavailability, adverse effects, and long-term safety have encouraged the exploration of alternative approaches aimed at stimulating endogenous neurosteroid production (Reddy, 2022) .

Among these approaches, naturally derived compounds have attracted considerable attention because of their diverse biological activities and favorable safety profiles. In addition to their established antioxidant, anti-inflammatory, and anti-apoptotic properties, increasing research has begun to explore their ability to interact with pathways involved in neurosteroid biosynthesis. Although the mechanisms vary among compounds, emerging findings suggest that certain phytochemicals may influence neurosteroidogenic processes, potentially contributing to their overall neuroprotective effects.

The regulation of neurosteroidogenesis by natural compounds represents a novel therapeutic avenue for combating neurodegenerative processes. By promoting endogenous neurosteroid production, these agents may help preserve neuronal integrity, enhance synaptic function, mitigate neuroinflammation, and improve resistance to oxidative and metabolic stress. Consequently, increasing research efforts have focused on identifying natural products capable of targeting neurosteroidogenic pathways and evaluating their potential application in neurodegenerative disorders.

## 4.1 Ginsenoside Rb1

Ginsenoside Rb1 is one of the major bioactive constituents isolated from Panax ginseng, a medicinal plant that has been used for centuries in traditional Asian medicine. Among the numerous ginsenosides identified in ginseng, Rb1 is considered one of the most pharmacologically active compounds and has attracted considerable attention because of its neuroprotective, anti-inflammatory, antioxidant, and anti-apoptotic properties. These diverse biological activities have led to increasing interest in the potential application of ginsenoside Rb1 in neurological and neurodegenerative disorders (Lng et al., 2024) .

Ginsenoside Rb1 is a dammarane-type triterpenoid saponin belonging to the protopanaxadiol (PPD) group of ginsenosides. Its structure consists of a hydrophobic tetracyclic dammarane aglycone backbone linked to multiple sugar moieties, primarily β-D-glucose residues attached at the C-3 and C-20 positions. The presence of these carbohydrate groups contributes significantly to its physicochemical characteristics and biological activities. The molecular formula of ginsenoside Rb1 is $C_{54}H_{92}O_{23}$, and its molecular weight is approximately 1109.29 g/mol. Due to its large molecular size and high degree of glycosylation, ginsenoside Rb1 exhibits high aqueous solubility but relatively low membrane permeability, which contributes to its limited oral bioavailability. Following oral administration, intestinal microbiota can metabolize ginsenoside Rb1 into smaller and more absorbable metabolites, such as compound K, thereby enhancing its biological activity and systemic availability (Kim et al., 2011) . The chemical structure of Ginsenoside Rb1is presented in Figure 8.

Structurally, the dammarane skeleton of ginsenoside Rb1 resembles the steroid nucleus found in endogenous steroid hormones, although it possesses distinct glycosidic substitutions that differentiate it from mammalian steroids. This steroid-like framework has been proposed to contribute to its interactions with multiple cellular signaling pathways involved in neuronal survival, oxidative stress regulation, and neuroprotection. The amphiphilic nature of the molecule, resulting from the coexistence of hydrophobic and hydrophilic domains, enables ginsenoside Rb1 to interact with cellular membranes and intracellular signaling targets, thereby influencing diverse physiological processes.

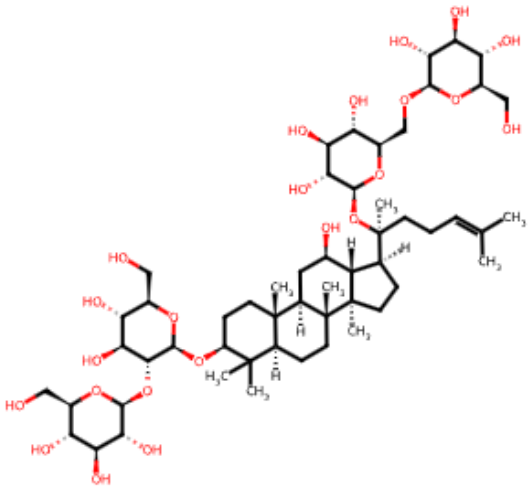


**Figure 1.** Chemical structure of Ginsenoside Rb1

Evidence for the involvement of ginsenoside Rb1 in neurosteroid regulation was provided by Lee and colleagues, who investigated the effects of Rb1 on brain neurosteroid levels in a mouse model of acute immobilization stress. The study demonstrated that exposure to acute stress significantly increased the concentrations of both THP and DHP in the frontal cortex and cerebellum, indicating that neurosteroid metabolism is responsive to psychological stress. Notably, oral administration of ginsenoside Rb1 (10 mg/kg) prior to stress exposure significantly attenuated the stress-induced elevation of THP levels in both brain regions. In addition, DHP levels were significantly reduced in the cerebellum compared with stressed animals that did not receive Rb1 treatment. These findings indicate that ginsenoside Rb1 can modulate endogenous neurosteroid responses triggered by stress. The authors proposed that the observed effects may result from alterations in neurosteroid metabolism and the neuroendocrine mechanisms activated during stress. By attenuating excessive stress-induced increases in neurosteroid levels, ginsenoside Rb1 may contribute to the maintenance of neurochemical homeostasis and the regulation of adaptive responses within the central nervous system. Furthermore, these findings suggest that modulation of neurosteroid pathways may represent an additional mechanism underlying the neuroprotective properties of ginsenoside Rb1.

Although the precise molecular mechanisms remain to be fully elucidated, the ability of ginsenoside Rb1 to influence the levels of progesterone-derived neurosteroids highlights its potential as a natural modulator of neurosteroidogenesis. This observation is of particular

interest given the growing evidence implicating neurosteroid dysregulation in the pathogenesis of several neurological and neurodegenerative disorders (Lee et al., 2006).

## 4.2 Palmitoylethanolamide (PEA)

Palmitoylethanolamide (PEA) is an endogenous fatty acid ethanolamide naturally produced in various tissues of the human body, including the central nervous system, in response to cellular stress and inflammation. In addition to its endogenous production, PEA is naturally present in several food sources, including egg yolk, soybeans, peanuts, soy lecithin, and other legumes. It belongs to a family of bioactive lipid mediators known as N-acylethanolamines and is structurally related to endocannabinoids, although it does not directly bind to classical cannabinoid receptors. Over the past decades, PEA has attracted considerable scientific interest owing to its potent anti-inflammatory, analgesic, neuroprotective, and immunomodulatory properties. Furthermore, PEA demonstrates a favorable safety profile and is currently utilized as a nutraceutical and dietary supplement for the management of several inflammatory and neurological conditions (Clayton et al., 2021).

PEA is an endogenous lipid molecule with the molecular formula $C_{18}H_{37}NO_2$ and a molecular weight of approximately 299.49 g/mol. Structurally, it consists of palmitic acid linked to ethanolamine through an amide bond, forming a highly lipophilic molecule capable of readily interacting with cellular membranes. Although not a steroid molecule, PEA exerts important regulatory effects on neurosteroidogenic pathways through activation of the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-α), a key modulator of inflammation, cellular metabolism, and neurosteroid synthesis. The chemical structure of PEA is presented in Figure 9.

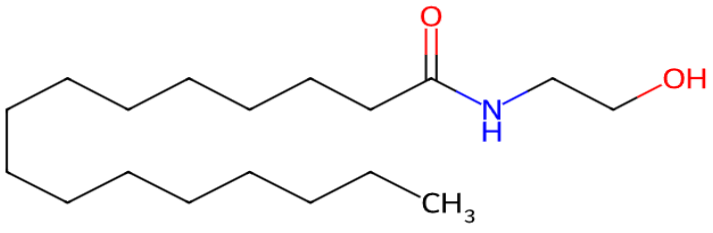



**Figure 2.** Chemical structure of Palmitoylethanolamide (PEA)

Among the natural compounds investigated for their effects on neurosteroidogenesis, PEA is one of the most extensively characterized. Evidence supporting its neurosteroidogenic activity

was provided by Mattace Raso and colleagues, who demonstrated that PEA stimulates de novo neurosteroid synthesis in astrocytes through activation of PPAR-α. In both C6 glioma cells and primary murine astrocytes, PEA treatment significantly increased the expression of steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage enzyme (P450scc), two proteins that catalyze critical early steps in neurosteroid biosynthesis. Importantly, PEA treatment also resulted in a significant increase in the production of allopregnanolone (ALLO), one of the most potent neuroprotective neurosteroids. The effects of PEA were abolished following pharmacological inhibition or genetic silencing of PPAR-α, confirming the involvement of this receptor in mediating PEA-induced neurosteroidogenesis (Raso et al., 2011).

Further evidence was obtained in experimental models of acute and persistent pain, where PEA administration significantly increased spinal cord allopregnanolone levels and restored the expression of StAR and P450scc. Notably, inhibition of neurosteroid synthesis partially reduced the biological effects of PEA, indicating that enhanced neurosteroid production contributes directly to its neuroprotective and analgesic actions. These findings provide strong evidence that PEA regulates de novo neurosteroid synthesis and that neurosteroidogenesis represents an important mechanism underlying its therapeutic effects (Sasso et al., 2012).

Additional support for the interaction between PEA and neurosteroid pathways was provided by studies demonstrating that PEA normalizes brain allopregnanolone levels and upregulates neurosteroidogenic enzyme expression in animal models characterized by impaired neurosteroidogenesis. These effects were associated with improved behavioral outcomes, including reduced anxiety- and depression-like behaviors, further highlighting the functional significance of PEA-induced neurosteroid synthesis (Locci & Pinna, 2019).

Collectively, the available evidence identifies PEA as a promising natural regulator of neurosteroidogenesis. Through activation of PPAR-α and subsequent upregulation of key steroidogenic proteins, PEA enhances allopregnanolone biosynthesis and promotes neuroprotective responses within the nervous system. Given the established involvement of neurosteroid dysregulation in numerous neurological and neurodegenerative disorders, PEA represents a particularly attractive candidate for strategies aimed at restoring neurosteroid homeostasis and improving neuronal resilience.

## 4.3 L-Ascorbic Acid (Vitamin C)

L-ascorbic acid, commonly known as vitamin C, is a water-soluble vitamin that is widely distributed in fruits and vegetables and is essential for normal physiological function. Beyond its well-established role as an antioxidant, vitamin C participates in numerous biochemical processes within the central nervous system, including neurotransmitter synthesis, neuronal maturation, and protection against oxidative stress. Increasing evidence indicates that vitamin C may also influence neurosteroid metabolism, suggesting an additional mechanism through which it contributes to brain function and neuroprotection (Gęgotek & Skrzydlewska, 2022) .

Vitamin C possesses the molecular formula $C_6H_8O_6$ and a molecular weight of approximately 176.12 g/mol. Structurally, it is a six-carbon lactone derived from glucose and is characterized by strong reducing properties that enable it to function as an effective antioxidant. The chemical structure of vitamin C is presented in Figure 10.

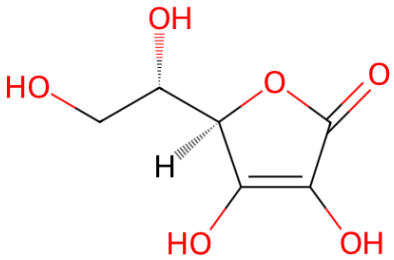



**Figure 3.** Chemical structure of L-ascorbic acid (Vitamin C)

Evidence supporting a role for vitamin C in neurosteroidogenesis was provided by studies investigating the regulation of neurosteroid synthesis in rat brain tissue. Treatment with L-ascorbic acid produced significant increases in the levels of several neurosteroids, including pregnenolone, dehydroepiandrosterone (DHEA), and allotetrahydrodeoxycorticosterone (THDOC) in the cerebral cortex. Regional differences were observed, with vitamin C additionally increasing progesterone and allopregnanolone concentrations in the hippocampus, while selectively increasing progesterone levels in the striatum. These findings demonstrate that vitamin C can modulate the synthesis and accumulation of neurosteroids within specific brain regions (Roscetti et al., 1998) .

The ability of vitamin C to increase multiple neurosteroids is particularly noteworthy because compounds such as pregnenolone, DHEA, progesterone, and allopregnanolone play important roles in neuronal survival, synaptic plasticity, stress adaptation, and neuroprotection. By

enhancing the production of these neuroactive steroids, vitamin C may contribute to the maintenance of neural homeostasis and support resistance to neurodegenerative processes. Collectively, the available evidence suggests that vitamin C acts not only as an antioxidant but also as a modulator of neurosteroid synthesis. Experimental studies indicate that L-ascorbic acid promotes the formation and accumulation of several neuroprotective neurosteroids, highlighting its potential relevance in strategies aimed at restoring neurosteroid homeostasis in neurological and neurodegenerative disorders.

# CHAPTER 5. Discussion

Neurodegenerative diseases are characterized by progressive neuronal dysfunction and loss, resulting in significant cognitive, motor, and behavioral impairments. Despite differences in their clinical manifestations and pathological features, Alzheimer's disease, Parkinson's disease, multiple sclerosis, Huntington's disease and amyotrophic lateral sclerosis share several common mechanisms, including oxidative stress, neuroinflammation, mitochondrial dysfunction, and impaired neuronal survival. In recent years, growing evidence has suggested that alterations in neurosteroid homeostasis may represent an additional and important contributor to the pathophysiology of these disorders. The findings reviewed in this thesis indicate that disturbances in neurosteroid synthesis and metabolism are consistently observed across multiple neurodegenerative conditions, highlighting the potential importance of neurosteroidogenic pathways in maintaining neuronal integrity and function.

Neurosteroids exert numerous physiological effects within the nervous system, including modulation of neurotransmission, regulation of synaptic plasticity, promotion of myelination, and protection against inflammatory and oxidative damage. Among these compounds, allopregnanolone has received particular attention because of its potent neuroprotective properties and its ability to positively modulate GABA-A receptor activity. Evidence discussed in Chapter 3 demonstrated that reductions in allopregnanolone and other neuroactive steroids have been reported in several neurodegenerative diseases. Similar alterations have also been observed for pregnenolone, progesterone, dehydroepiandrosterone (DHEA), and related metabolites. These changes suggest that impaired neurosteroidogenesis may contribute to a loss of endogenous neuroprotective mechanisms, thereby increasing susceptibility to neuronal injury and accelerating disease progression.

Given the emerging role of neurosteroid dysregulation in neurodegeneration, considerable interest has developed in strategies capable of restoring neurosteroid homeostasis. While direct administration of neurosteroids has produced encouraging results in several experimental models, limitations related to pharmacokinetics, bioavailability, and long-term safety have encouraged the exploration of alternative approaches that stimulate endogenous neurosteroid production. In this context, naturally occurring compounds have attracted increasing attention

because they often possess favorable safety profiles and can modulate multiple biological pathways simultaneously (Reddy, 2022).

Among the compounds examined in this thesis, ginsenoside Rb1 demonstrated the ability to influence neurosteroid metabolism by attenuating stress-induced changes in allopregnanolone and 5α-dihydroprogesterone levels. Although the precise molecular mechanisms underlying these effects remain incompletely understood, the available findings suggest that modulation of endogenous neurosteroid responses may contribute to the neuroprotective properties that have been attributed to this compound. The ability of ginsenoside Rb1 to regulate neurosteroid levels under conditions of stress is particularly relevant, as chronic stress and neuroendocrine dysregulation have been implicated in the progression of several neurodegenerative disorders (Lee et al., 2006; Ling et al., 2024).

Palmitoylethanolamide (PEA) exhibited the strongest evidence for direct regulation of neurosteroidogenesis among the compounds investigated. Experimental studies demonstrated that PEA stimulates neurosteroid synthesis through activation of PPAR-α, resulting in increased expression of steroidogenic proteins such as StAR and P450scc and enhanced production of allopregnanolone. These findings are particularly significant because they establish a mechanistic relationship between a naturally occurring bioactive compound and the neurosteroidogenic machinery itself. Furthermore, the observation that inhibition of neurosteroid synthesis attenuates some of the biological effects of PEA suggests that increased neurosteroid production is not merely an associated phenomenon but may represent a key mechanism underlying its neuroprotective activity (Clayton et al., 2021; Locci & Pinna, 2019; Raso et al., 2011; Sasso et al., 2012).

Vitamin C also demonstrated a remarkable ability to modulate neurosteroid synthesis and accumulation within the brain. Experimental studies showed that L-ascorbic acid increases the levels of several neurosteroids, including pregnenolone, DHEA, progesterone, allopregnanolone, and THDOC. Unlike ginsenoside Rb1, which primarily modulated specific neurosteroid responses, or PEA, which strongly stimulated allopregnanolone biosynthesis, vitamin C appeared to exert broader effects on multiple neurosteroid pathways. These findings suggest that vitamin C may contribute to neuroprotection through mechanisms extending beyond its well-established antioxidant activity. The ability to simultaneously influence

multiple neuroactive steroids may be particularly advantageous in disorders characterized by widespread disturbances in neurosteroid metabolism (Roscetti et al., 1998) .

Although the three compounds reviewed differ substantially in their chemical structures and primary biological activities, all share the capacity to influence neurosteroid homeostasis. This observation supports the concept that neurosteroidogenesis can be modulated through multiple pathways and molecular targets. Some compounds may act directly on the steroidogenic machinery, whereas others may influence signaling networks that regulate neurosteroid production or metabolism. Regardless of the specific mechanism involved, restoration of neurosteroid balance appears to represent a common outcome that may contribute significantly to neuroprotection.

Nevertheless, the overall body of evidence reviewed in this thesis supports the hypothesis that neurosteroidogenesis represents a promising therapeutic target in neurodegenerative diseases. Natural compounds capable of enhancing endogenous neurosteroid production may offer a novel and physiologically relevant strategy for restoring neuronal homeostasis and counteracting neurodegenerative processes. Future research should focus on identifying additional neurosteroidogenic compounds, clarifying their molecular mechanisms of action, and evaluating their clinical efficacy in patients with neurodegenerative disorders. Improved understanding of these pathways may ultimately facilitate the development of innovative therapeutic approaches aimed at preserving neuronal function and slowing disease progression.

Despite the encouraging findings presented throughout this thesis, significant knowledge gaps remain regarding the therapeutic application of neurosteroidogenic natural compounds. Current evidence is largely derived from preclinical studies, and the translation of these findings into clinical practice remains limited. While compounds such as ginsenoside Rb1, palmitoylethanolamide, and vitamin C have demonstrated the capacity to modulate neurosteroid levels and neurosteroidogenic pathways, additional studies are required to clarify their precise molecular mechanisms of action, optimal dosing regimens, long-term efficacy, and therapeutic relevance in different neurodegenerative disorders. Future research should also focus on identifying novel natural compounds capable of enhancing neurosteroidogenesis and characterizing their interactions with key steroidogenic regulators, including StAR, TSPO, CYP11A1, 5α-reductase, and 3α-hydroxysteroid dehydrogenase.

Furthermore, although these compounds are generally regarded as safe and well tolerated, important questions regarding their long-term safety profiles remain unresolved. The full spectrum of potential adverse effects, contraindications, and interactions with conventional pharmacological therapies has not yet been comprehensively investigated, particularly in elderly individuals and patients with neurodegenerative diseases who frequently receive multiple medications. Consequently, additional toxicological, pharmacokinetic, and clinical studies are necessary to establish the long-term safety, tolerability, and efficacy of neurosteroidogenic natural compounds before their widespread clinical application can be recommended.

**Neurosteroid dysregulation as a common feature of neurodegenerative diseases.**

Among the neurosteroids investigated, allopregnanolone has received particular attention because of its ability to positively modulate GABA-A receptors and influence neuronal excitability, stress responses, neuroinflammatory processes, and neuronal survival. Its biological relevance extends beyond neurotransmission, as experimental evidence suggests that allopregnanolone may also contribute to mechanisms involved in cellular protection and tissue repair. The findings discussed in Chapter 3 indicate that alterations in allopregnanolone concentrations occur in several neurodegenerative disorders. In Alzheimer's disease, reduced allopregnanolone levels have been observed in affected brain regions and have been associated with increasing neuropathological severity. These observations suggest that impaired availability of this neurosteroid may accompany the progression of neurodegenerative processes. Nevertheless, the simultaneous elevation of other steroids, including pregnenolone and DHEA, indicates that neurosteroid metabolism is not uniformly suppressed.

Such differences may reflect compensatory responses, altered enzymatic conversion, or disease-associated changes in the cellular populations responsible for steroid production. Importantly, the available evidence does not establish whether these alterations contribute directly to neuronal degeneration or arise because of the pathological process.

Similar considerations apply to Parkinson's disease. Reduced concentrations of allopregnanolone and 5α-dihydroprogesterone have been reported in biological samples from patients with PD, together with changes in the expression of enzymes involved in neurosteroid metabolism (di Michele et al., 2003; Luchetti et al., 2010). These findings are particularly relevant because dopaminergic neurodegeneration is accompanied by disturbances in GABAergic neurotransmission and mitochondrial function.

The reduction of specific progesterone-derived neurosteroids could therefore contribute to altered neuronal excitability and reduced endogenous neuroprotection. However, further investigations are necessary to establish whether these changes directly influence dopaminergic neuronal survival or primarily reflect secondary adaptations to nigrostriatal degeneration.

Multiple sclerosis provides additional evidence supporting the relationship between neurosteroid metabolism and neuroinflammation. Studies examining central nervous system tissue and biological fluids have identified alterations in allopregnanolone, pregnenolone, DHEA, and other neuroactive steroids, together with changes in steroidogenic enzyme expression (Noorbakhsh et al., 2011; Caruso et al., 2014).

Of particular interest is the observation that neurosteroid profiles may vary according to disease activity, inflammatory status, and sex. These findings suggest that neurosteroid regulation is closely associated with the inflammatory microenvironment and may be influenced by the progression of demyelinating processes.

In this context, the biological activities of progesterone-derived neurosteroids may be especially relevant because they have been implicated in oligodendrocyte function, myelin maintenance, and neuroimmune regulation. Nevertheless, the relationship between neurosteroid alterations and remyelination remains insufficiently characterized in humans.

More recently, reduced concentrations of several neurosteroids have also been reported in the cerebrospinal fluid of patients with ALS (Lucchi et al., 2024). The inverse relationship observed between neurosteroid concentrations and neurofilament levels suggests a possible association with neuronal injury. However, the absence of a consistent relationship with clinical progression or survival limits their current interpretation as prognostic biomarkers.

Taken together, these findings indicate that disturbances in neurosteroid homeostasis occur in several neurodegenerative diseases. However, the direction and magnitude of these changes depend on the specific neurosteroid, the biological compartment investigated, and the pathological condition. Therefore, the available evidence supports the relevance of neurosteroid dysregulation but does not establish a single neurosteroid profile common to all neurodegenerative disorders.

**Molecular mechanisms underlying the regulation of neurosteroidogenesis**

The therapeutic potential of neurosteroid modulation is closely related to the complexity of steroidogenic pathways within the central nervous system.

Neurosteroid synthesis involves several sequential biochemical reactions, beginning with the availability of cholesterol and its conversion into pregnenolone by the mitochondrial enzyme CYP11A1. Subsequent enzymatic transformations generate progesterone and other neuroactive metabolites, including 5α-dihydroprogesterone and allopregnanolone.

These reactions are regulated by several factors, including cholesterol availability, mitochondrial function, steroidogenic enzyme expression, and the metabolic activity of neurons and glial cells.

The steroidogenic acute regulatory protein (StAR) plays an established role in facilitating mitochondrial cholesterol transport in classical steroidogenic tissues. However, the regulation of steroid synthesis within the nervous system may involve additional mechanisms that vary according to cell type and physiological conditions.

The mitochondrial translocator protein TSPO has historically been considered an important regulator of cholesterol transport and steroidogenesis. Its localization in the outer mitochondrial membrane and its association with inflammatory and mitochondrial responses have contributed to considerable interest in its potential pharmacological modulation.

Nevertheless, the precise role of TSPO in steroid biosynthesis remains controversial. Studies using genetically modified experimental models have challenged the hypothesis that TSPO is universally required for steroidogenesis, indicating that its contribution may be context-dependent or less direct than previously proposed (Selvaraj et al., 2015) . Other investigations have supported its involvement under experimental conditions.

Consequently, changes in TSPO expression or activity should not automatically be interpreted as evidence of increased or decreased neurosteroid production.

This distinction is particularly important when evaluating natural compounds that influence mitochondrial function or inflammatory signaling. A compound may modify TSPO expression, reduce oxidative stress, or improve mitochondrial integrity without necessarily exerting a direct effect on steroid biosynthesis.

Similarly, an increase in neurosteroid concentrations does not necessarily demonstrate enhanced de novo synthesis, since it may also result from reduced degradation, altered metabolic conversion, or changes in steroid transport.

A more comprehensive evaluation of neurosteroidogenic activity should therefore combine quantitative steroid measurements with the investigation of relevant enzymes, mitochondrial function, and metabolic pathways.

**Comparative evaluation of ginsenoside Rb1, palmitoylethanolamide, and vitamin C**

The three natural compounds examined in this thesis provide interesting examples of different approaches to neurosteroid modulation.

Ginsenoside Rb1 is a triterpenoid saponin derived from Panax ginseng, characterized by antioxidant, anti-inflammatory, and anti-apoptotic activities. The experimental findings discussed in Chapter 4 indicate that Rb1 can influence stress-induced changes in the concentrations of progesterone-derived neurosteroids.

Administration of Rb1 attenuated the increase in allopregnanolone and 5α-dihydroprogesterone observed following acute immobilization stress in mice (Lee et al., 2006).

This observation deserves particular attention because it demonstrates that the biological action of a neurosteroid-modulating compound does not necessarily consist of increasing neurosteroid concentrations.

Under stressful conditions, the elevation of certain neurosteroids may represent an adaptive response aimed at counteracting excessive neuronal activation. The ability of Rb1 to attenuate this response may reflect a modification of stress-related neuroendocrine signaling rather than a direct inhibition of steroidogenesis.

Therefore, Rb1 should be considered a potential regulator of neurosteroid homeostasis rather than simply a stimulator of neurosteroid production.

An important limitation is that the available findings were obtained in an acute stress model, which differs substantially from the chronic and multifactorial pathological processes underlying neurodegenerative diseases. Further studies are required to determine whether comparable effects occur in experimental models of Alzheimer's or Parkinson's disease and whether neurosteroid modulation contributes directly to the neuroprotective actions of Rb1.

Palmitoylethanolamide represents a different pharmacological approach. Unlike ginsenoside Rb1, PEA is an endogenous lipid mediator belonging to the N-acylethanolamine family and is involved in the regulation of inflammatory and cellular stress responses.

Its biological effects have been associated with several molecular pathways, particularly PPAR-α signaling, and with the modulation of neuroimmune responses.

The potential relationship between PEA and neurosteroidogenesis is especially interesting because neuroinflammation and steroid metabolism are closely interconnected.

Activated microglia and astrocytes contribute to the production of inflammatory mediators, reactive oxygen species, and other factors capable of modifying neuronal and glial function. These processes may also influence the expression or activity of enzymes involved in neurosteroid synthesis and metabolism.

By reducing inflammatory signalling and improving cellular homeostasis, PEA could potentially create conditions favourable to the maintenance or restoration of endogenous neurosteroid pathways.

However, the contribution of neurosteroidogenesis to the overall neuroprotective activity of PEA requires further investigation. Its anti-inflammatory and neuroprotective effects cannot be attributed exclusively to changes in neurosteroid production.

The possibility that PEA acts through multiple interconnected mechanisms may nevertheless represent a pharmacological advantage in neurodegenerative diseases, where neuronal injury results from the simultaneous activation of several pathological pathways.

Vitamin C differs from both ginsenoside Rb1 and PEA because of its essential physiological role as a water-soluble antioxidant and enzymatic cofactor.

Within the central nervous system, vitamin C contributes to the regulation of oxidative balance and participates in several metabolic processes important for neuronal function.

The findings reviewed in Chapter 4 suggest that L-ascorbic acid may also influence the concentrations of selected neuroactive steroids, including pregnenolone, progesterone, DHEA, and allopregnanolone.

These observations raise the possibility that vitamin C may contribute to neuroprotection through mechanisms extending beyond its antioxidant activity.

Nevertheless, the relationship between vitamin C and neurosteroidogenesis requires careful interpretation. Changes in steroid concentrations observed under experimental conditions do not necessarily indicate that vitamin C directly activates steroidogenic enzymes.

Its effects may also be mediated by improvements in cellular redox status, mitochondrial function, or the metabolic environment supporting steroid synthesis.

Furthermore, vitamin C is physiologically regulated within the central nervous system, and its effects may depend on baseline nutritional status, cellular concentrations, and experimental conditions.

Consequently, evidence of steroid modulation in experimental systems cannot be directly extrapolated to the therapeutic effects of vitamin C supplementation in patients with neurodegenerative diseases.

Overall, the comparison among these compounds suggests that neurosteroid homeostasis can potentially be influenced through different biological mechanisms. Ginsenoside Rb1 appears to modify stress-associated neurosteroid responses, PEA may influence neurosteroid-related pathways through neuroimmune and metabolic regulation, while vitamin C may affect steroid homeostasis in association with its antioxidant and metabolic activities.

However, the strength of the evidence differs among the compounds, and further mechanistic studies are required before their actions can be considered equivalent.

**Therapeutic implications and challenges in clinical translation**

The potential therapeutic relevance of natural compounds capable of modulating neurosteroidogenesis must be considered in relation to the multifactorial nature of neurodegenerative diseases.

Unlike pharmacological approaches directed toward a single molecular target, compounds with pleiotropic biological activities may influence several interconnected mechanisms involved in neuronal injury. These include inflammatory signaling, mitochondrial dysfunction, oxidative stress, and alterations in neurosteroid metabolism.

This characteristic may be particularly relevant in neurodegenerative disorders, where different pathological processes interact and reinforce one another during disease progression.

Nevertheless, the simultaneous modulation of multiple biological pathways also introduces considerable challenges in identifying the mechanisms responsible for therapeutic effects.

An important consideration concerns the relationship between neurosteroid synthesis and the different cellular populations of the central nervous system.

Neurons, astrocytes, microglia, and oligodendrocytes participate in maintaining the biochemical environment required for neuronal survival. However, their contributions to steroid metabolism and their responses to neuroactive steroids differ considerably.

Astrocytes are particularly interesting because they participate in metabolic support, inflammatory regulation, and the synthesis or transformation of neuroactive compounds. Experimental evidence indicating that PEA can stimulate neurosteroidogenic pathways in astrocytic models suggests that glial cells may represent important targets for pharmacological interventions aimed at restoring neurosteroid homeostasis.

Similarly, the relationship between neurosteroids and microglial activation deserves further investigation. Chronic microglial activation contributes to the maintenance of neuroinflammatory processes in several neurodegenerative disorders, while neuroactive steroids may influence inflammatory signaling and glial responses.

Therefore, compounds capable of simultaneously reducing neuroinflammation and modulating neurosteroid metabolism could potentially exert complementary protective effects.

However, demonstrating such interactions requires experimental approaches that distinguish direct effects on steroidogenic pathways from secondary changes resulting from reduced cellular stress or inflammation.

Another important issue concerns the pharmacokinetic properties of the compounds investigated.

Ginsenoside Rb1 exhibits limited oral bioavailability and membrane permeability, partly because of its relatively large molecular size and glycosylated structure. Its metabolism by the intestinal microbiota may generate compounds with different absorption profiles and biological activities.

Consequently, the neurosteroid-modulating effects observed in experimental studies may depend not only on the parent compound but also on its metabolites and their ability to reach the central nervous system.

PEA presents different formulation challenges because of its lipophilic nature and limited aqueous solubility. These characteristics may influence dissolution, absorption, and systemic exposure following oral administration.

The development of micronized, ultramicronized, or lipid-based formulations may offer opportunities to improve its pharmaceutical performance, although improved formulation properties do not necessarily establish enhanced central nervous system exposure or greater clinical efficacy.

Vitamin C, in contrast, is characterized by specific transport and homeostatic mechanisms that regulate its distribution within the central nervous system. Therefore, increasing systemic vitamin C intake does not necessarily produce a proportional increase in cerebral concentrations or neurosteroid synthesis.

These considerations emphasize the importance of investigating the relationship between administered dose, systemic exposure, brain availability, and pharmacodynamic effects.

The blood–brain barrier represents an additional challenge for the development of neurosteroid-modulating therapies. Effective pharmacological intervention requires either sufficient exposure of relevant central nervous system targets or the activation of peripheral mechanisms capable of influencing cerebral homeostasis.

Future investigations should therefore evaluate the distribution of these compounds and their metabolites, together with their ability to modulate neurosteroid concentrations in specific brain regions.

Innovative pharmaceutical formulations, including lipid-based delivery systems and other nanotechnology approaches, may provide useful strategies for improving the delivery of selected compounds. However, their effectiveness and safety must be established through appropriate experimental studies.

**Limitations of the available evidence and future research directions**

Despite the promising findings discussed throughout this thesis, several limitations must be acknowledged.

First, most studies investigating the relationship between natural compounds and neurosteroidogenesis have been conducted in cellular systems or animal models. Although these experimental approaches provide valuable mechanistic information, they do not fully reproduce the complexity of human neurodegenerative diseases.

Second, the experimental conditions used to investigate neurosteroid modulation differ considerably among studies. Variations in compound concentration, administration route, treatment duration, biological model, and analytical methodology make direct comparisons difficult.

Furthermore, neurosteroid concentrations may vary according to brain region, sex, age, hormonal status, and pathological conditions. These variables should be carefully considered when interpreting experimental results and designing future investigations.

A third limitation concerns the difficulty of establishing a causal relationship between neurosteroid modulation and neuroprotection.

The observation that a compound simultaneously increases neurosteroid concentrations and improves neuronal survival does not necessarily demonstrate that neurosteroid synthesis mediates its protective activity.

More conclusive evidence requires experimental approaches involving pharmacological inhibition, genetic manipulation of steroidogenic enzymes, or interference with specific neurosteroid signaling pathways.

In this respect, the studies investigating PEA provide particularly relevant mechanistic information because they have examined the involvement of PPAR-α and steroidogenic proteins in its biological effects.

Nevertheless, additional investigations are needed to determine whether these mechanisms contribute to neuroprotection in chronic neurodegenerative conditions.

Future research should also incorporate comprehensive analytical approaches capable of simultaneously quantifying multiple neurosteroids and their metabolites.

Techniques such as liquid chromatography coupled with tandem mass spectrometry could provide detailed steroid profiles and facilitate the identification of disease-specific alterations in neurosteroid metabolism.

Combining these measurements with the analysis of steroidogenic enzyme expression, mitochondrial function, inflammatory mediators, and neuronal survival markers would allow a more integrated evaluation of the mechanisms involved.

Attention should be directed toward the relationship between neurosteroidogenesis and mitochondrial dysfunction, since mitochondria participate in the initial steps of steroid biosynthesis and are simultaneously major targets of oxidative and metabolic damage in neurodegenerative diseases.

The identification of compounds capable of preserving mitochondrial integrity while supporting appropriate neurosteroid metabolism may therefore represent an interesting direction for future pharmacological research.

Another important objective is the development of experimental models that better reproduce the interactions among neurons and glial cells.

Co-culture systems, human induced pluripotent stem cell-derived neural models, and more complex cellular platforms could provide useful tools for investigating how natural compounds influence neurosteroid metabolism under conditions of chronic inflammation and neurodegenerative stress.

Finally, clinical investigations are essential to establish whether the modulation of neurosteroidogenesis translates into measurable therapeutic benefits.

Such studies should evaluate not only clinical outcomes but also pharmacokinetic parameters, neurosteroid profiles, biomarkers of neuronal injury, and possible interactions with conventional treatments.

Long-term safety is particularly important because neurodegenerative diseases predominantly affect older individuals, who frequently present comorbidities and receive multiple medications. Although naturally occurring compounds are often perceived as relatively safe, their natural origin does not guarantee the absence of adverse effects, pharmacological interactions, or dose-dependent toxicity.

Therefore, the development of neurosteroid-modulating compounds as therapeutic agents requires rigorous evaluation of their efficacy, safety, and mechanisms of action.

**Overall interpretation**

The evidence examined in this thesis supports the hypothesis that neurosteroid homeostasis represents an important component of central nervous system physiology and may be altered during neurodegenerative processes.

The comparison among ginsenoside Rb1, palmitoylethanolamide, and vitamin C highlights the diversity of mechanisms through which naturally occurring compounds may influence neurosteroid metabolism.

Among these compounds, PEA appears to provide the most direct experimental evidence of stimulation of neurosteroidogenic pathways, particularly through PPAR-α-dependent mechanisms involving steroidogenic proteins and allopregnanolone production.

Ginsenoside Rb1 and vitamin C also demonstrate potentially relevant effects on neurosteroid concentrations, although their underlying mechanisms and therapeutic implications remain less clearly established.

Importantly, these findings indicate that the pharmacological regulation of neurosteroid homeostasis should not be considered exclusively in terms of increasing neurosteroid

production. Depending on the pathological context, beneficial effects may result from restoring physiological steroid profiles, regulating specific metabolic pathways, or preventing inappropriate neurosteroid responses.

Thus, neurosteroidogenesis represents a potentially valuable therapeutic target, but its modulation must be understood within the broader network of mitochondrial, inflammatory, and neurochemical processes involved in neurodegeneration.

Further mechanistic and clinical investigations are required to determine whether natural compounds capable of influencing these pathways can provide effective therapeutic benefits in patients with neurodegenerative diseases.

# CHAPTER 6. Conclusion

Neurosteroids represent an important class of endogenous bioactive molecules involved in the regulation of neuronal excitability, synaptic plasticity, neuroinflammation, myelination, and neuronal survival. Growing evidence indicates that alterations in neurosteroid synthesis, metabolism, and signaling are associated with several neurodegenerative diseases, suggesting that the maintenance of neurosteroid homeostasis may play a significant role in preserving central nervous system function.

The literature reviewed in this thesis highlights the involvement of neurosteroid dysregulation in Alzheimer's disease, Parkinson's disease, multiple sclerosis, and amyotrophic lateral sclerosis. Alterations in the concentrations of allopregnanolone, pregnenolone, progesterone, dehydroepiandrosterone (DHEA), and their metabolites have been reported in both clinical investigations and experimental models. These findings suggest that disturbances in neurosteroidogenic pathways may contribute to neuronal vulnerability by affecting inhibitory neurotransmission, inflammatory responses, and endogenous neuroprotective mechanisms. However, the available evidence does not yet establish whether these alterations represent primary contributors to neurodegeneration, secondary consequences of disease progression, or compensatory responses to neuronal injury.

Within this context, the modulation of endogenous neurosteroidogenesis has emerged as a promising pharmacological approach. Rather than relying exclusively on the administration of exogenous neurosteroids, strategies aimed at regulating their local synthesis may offer opportunities to influence physiological neuroprotective pathways. Natural compounds are of particular interest because of their diverse biological activities and their potential to interact with multiple molecular mechanisms involved in neuronal homeostasis.

Among the compounds examined in this thesis, ginsenoside Rb1, palmitoylethanolamide (PEA), and L-ascorbic acid (vitamin C) have demonstrated the ability to influence neurosteroid levels or neurosteroidogenic pathways through different mechanisms.

Ginsenoside Rb1 appears to modulate neurosteroid responses under stress-related conditions, particularly by attenuating alterations in allopregnanolone and 5α-dihydroprogesterone concentrations. These findings suggest that regulation of neurosteroid homeostasis may contribute to its biological effects, although the molecular mechanisms responsible for these changes require further investigation.

Palmitoylethanolamide represents a particularly interesting candidate because experimental evidence supports a more clearly defined relationship between its biological activity and the stimulation of neurosteroidogenesis. Through activation of peroxisome proliferator-activated receptor alpha (PPAR-α), PEA can promote the expression of proteins involved in steroid biosynthesis, including steroidogenic acute regulatory protein (StAR) and cytochrome P450 side-chain cleavage enzyme (P450scc/CYP11A1), thereby enhancing allopregnanolone production. This mechanism suggests that neurosteroidogenesis may contribute to the biological actions of PEA and provides a rationale for further investigation of its potential applications in neurological disorders.

Vitamin C has also demonstrated the capacity to influence neurosteroid metabolism, increasing the concentrations of several neuroactive steroids in experimental brain tissues. These observations suggest that its biological functions within the central nervous system may extend beyond its established antioxidant properties. Nevertheless, additional studies are necessary to determine the mechanisms underlying these effects and their relevance to neurodegenerative conditions.

Taken together, these findings indicate that natural compounds can influence neurosteroid homeostasis through distinct molecular pathways. However, their effects should not be considered equivalent, and the ability to increase neurosteroid concentrations does not necessarily translate into improved neurological outcomes. The biological consequences of neurosteroid modulation may depend on the specific steroid involved, its concentration, the affected brain region, and the pathological context.

An important limitation of the current evidence is its predominantly preclinical nature. Although experimental studies provide valuable insights into the molecular mechanisms regulating neurosteroidogenesis, direct evidence demonstrating that these natural compounds can prevent or slow neurodegenerative disease progression in humans remains insufficient. Furthermore, the relationship between changes in neurosteroid concentrations and clinically meaningful neuroprotective effects requires further clarification.

Future investigations should therefore focus on identifying the molecular targets involved in neurosteroidogenic regulation, establishing effective concentrations and dosing regimens, and evaluating the pharmacokinetic properties, central nervous system bioavailability, and long-term safety of these compounds. Attention should also be given to the development of

experimental models that reproduce the complex interactions among neurons, astrocytes, and microglial cells, as well as to the identification of reliable biomarkers capable of linking neurosteroid modulation to functional neurological outcomes.

From a pharmaceutical perspective, a better understanding of the interactions between natural compounds and neurosteroidogenic pathways could support the development of innovative therapeutic strategies, potentially complementary to existing treatments. However, rigorous clinical investigations will be essential to establish whether the promising biological effects observed experimentally can be translated into effective and safe interventions for patients with neurodegenerative diseases.

In conclusion, the evidence reviewed in this thesis identifies neurosteroidogenesis as an emerging and potentially valuable target for neuroprotective research. Natural compounds such as ginsenoside Rb1, PEA, and vitamin C provide interesting examples of how endogenous neurosteroid pathways may be pharmacologically modulated. Although their clinical potential remains to be established, further investigation of these mechanisms may contribute to the identification of novel therapeutic targets and the development of more effective approaches aimed at preserving neuronal function and limiting neurodegenerative processes.

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