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Progestogen-mediated neuroprotection in central nervous system disorders

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Abstract
Neuroactive steroids can be synthetic or endogenous molecules produced by neuronal and glial cells, and peripheral glands. Examples include estrogens, testosterone, progesterone and its reduced metabolites such as 5α-dihydroprogesterone and allopregnanolone. Steroids produced by neurons and glia target the nervous system and are called neurosteroids. Progesterone and analog molecules, known as progestogens, have been shown to exhibit neurotrophic, neuroprotective, antioxidant, anti-inflammatory, glial modulatory, promyelinating and remyelinating effects in several experimental models of neurodegenerative and injury conditions. Pleiotropic mechanisms of progestogens may act synergistically to prevent neuron degeneration, astrocyte and microglial reactivity, reducing morbidity and mortality. The aim of this review is to summarize the significant findings related to the actions of progesterone and other progestogens in experimental models and epidemiological and clinical trials of some of the most prevalent and debilitating chronic neurodegenerative disorders, namely Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis and Multiple Sclerosis. We evaluated progestogen alterations under pathological conditions, how pathology modifies their levels, as well as the intracellular mechanisms and glial interactions underlying their neuroprotective effects. Furthermore, an analysis of the potential of natural progestogens and synthetic progestins as neuroprotective and regenerative agents, when administered as hormone replacement therapy in menopause, is also discussed.
Keywords: Progesterone, allopregnanolone, neurodegenerative disease, neuroprotection.

1. Introduction

Sex steroid hormones play fundamental roles in reproductive biology and participate in other modulatory functions such as nervous system homeostasis. The gonads, adrenal glands and placenta mainly produce sex steroids. However, steroids are also synthesized *de novo* in the central nervous system (CNS) and peripheral nervous system (PNS) from cholesterol molecules by glia (oligodendrocytes and astrocytes in the CNS and Schwann cells in the PNS) and neurons (Figure 1). When produced in the CNS and PNS, they are called neurosteroids [1].

In the CNS, neurosteroids exert diverse functions, such as regulation of □-aminobutyric acid (GABA) and glutamate transmission, neuronal growth, brain development, synapse formation, myelination, cognition, neurogenesis, emotion, mood, dendritic growth, neuronal survival, reproductive and social behavior [1,2]. Neurosteroids correspond to a range of estrogens, androgens and progestogens, including 17β-estradiol (E2), testosterone, dihydrotestosterone (DHT), androstanediol, progesterone, allopregnanolone, and

dehydroepiandrosterone (DHEA) [2,3]. This review focuses on the role of specific neuroactive progestogens in neurodegenerative disorders.

Progesterone is the key regulator of the female cyclic reproductive tract. Changes in the levels of this hormone maintain pregnancy in all mammals. Interestingly, the physiological effects of progestogens on target cells are mediated by the classical (slow actions) and the non-classical (fast actions) pathways (Figure 2). The progesterone classical pathway is triggered by association in the cytoplasm and subsequent nuclear translocation of the classical progesterone receptors (PR): PR-A and PR-B, which are members of the nuclear receptor superfamily, that act as ligand-activated transcription factors [4,5]. The complex progesterone-PR binds to progesterone responsive elements (PRE) in the DNA, allowing the transcription of a specific set of genes [6]. The PR gene in humans is localized on chromosome 18, and it consists of 8 exons. The PR-A and PR-B isoforms are produced from the same gene transcript but are controlled by different promoters. Both isoforms have been identified in diverse tissues, including gonads, uterus, mammary glands, pancreas, bones, urinary tract and brain. PR-B acts as a transcription activator of progesterone responsive genes, while PR-A inhibits or counteracts PR-B activity, functioning as a modulator [7].

Concerning the non-classical pathway, it is triggered by membrane progesterone receptors (mPR) and progesterone receptor membrane components 1 and 2 (PGRMC1 and PGRMC2), which initiate rapid intracellular signaling cascades. The mPRs are metabotropic receptors composed of seven transmembrane domains which have been shown to activate G proteins. They belong to the progestin and adipoQ receptor (PAQR) family, and there are five known isoforms (mPRα, mPRβ, mPRγ, mPRδ and mPRε). Interestingly, mPRα is expressed in neurons and, following an insult, it has its expression increased in glial cells, suggesting a role in neuroinflammation [4]. Nonetheless, the functions and intracellular pathways activated by PGRMC1 and 2 have not been fully elucidated. Recent evidence suggests that PGRMC1 acts as an adaptor molecule for mPR, facilitating its translocation to the plasma membrane. In this sense, non-classical progesterone actions are proposed to be initiated by the PGRMC1-mPR complex and not the result of receptor action alone [8,9]. Stimulation of mPR triggers many quick gene transcription-independent responses accompanied by increased intracellular Ca^{2+} concentrations and second messenger activation [10]. Indeed, cAMP and mitogen activated protein kinase (MAPK) activation has been shown to promote CREB phosphorylation and regulate SRC2 co-activator activity [11]. Notably, these intracellular signaling cascades can trigger specific non-genomic responses or modulate genomic pathways [7]. For example, progesterone-mediated mPR activation leads to MAPK and protein kinase C (PKC) activation via cAMP, and mPRs also activate PI3K/Akt intracellular pathways. Simultaneously, phospholipase C (PLC) is activated, leading to intracellular Ca^{2+} store mobilization and increased cytosolic concentration of this cation [7,12].

It is relevant to point out that the hormonal activity of the neurosteroid allopregnanolone, an active metabolite of progesterone, was for a long time considered insignificant due to the lack of activity on classic PR. However, anti-seizure activity, mediated by positive $GABA_A$ receptor modulation, has been reported for this steroid (for a review on allopregnanolone synthesis, mechanisms and effects see Diviccaro et al [13]). Moreover, there is evidence that allopregnanolone and its analog ganaloxone act by binding to the neuronal cell mPRs and inhibits apoptosis [14]. Therefore, the combined $GABA_A$ modulation and the anti-apoptotic effect by mPRs stimulation respond for the complexity of allopregnanolone effects in the brain and will be discussed later in this review.

Regarding the regulation of production and release mechanisms, evidence supports that both progesterone and allopregnanolone increase during stress condition in healthy humans, with positive correlation between cortisol and progesterone levels. Progesterone has been especially linked to the willingness for social interaction, which supports the concept that allopregnanolone and progesterone release can ultimately lead to a decrease in anxiety and stress [15]. Moreover, the levels of the neuroesteroids DHEA, DHEA sulfate (DHEAS), and allopregnanolone decrease during aging, increasing neuron vulnerability to toxic agents, which is associated with neuronal apoptosis and degeneration [16]. Therefore, the production and release of these neuroactive steroids could provide protection against neuron dysfunction and apoptosis, whereas its gradual reduction would contribute to the aging process.

2. Progestogens in neurodegenerative disorders

Levels of neuroactive steroids, including progestogens, were shown to be altered under various experimental neurodegenerative conditions [17]. A variety of PRs are also expressed in both neurons and glia [12]. Interestingly, there is growing evidence that progesterone and other neurosteroids provide neuroprotection to the CNS and PNS through several mechanisms.

Moreover, neurosteroids are reported to have neuroinflammation modulatory properties [18] that may be attributed at least in part to suppression of microglial cell activation [19]. A previous study showed that allopregnanolone and progesterone reduce injury-induced expression of interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) at the mRNA and protein levels [20]. A recent study reported the allopregnanolone immunomodulatory effects at supraphysiologic levels (10 μM) in murine microglial cells. Allopregnanolone reduced microglial cell migration and phagocytic function [21]. These reports suggest that progestogens exert anti-inflammatory actions, at least in part, via down-regulation of glial pro-inflammatory cytokine gene expression. Besides the effects on glial cells, progesterone was also reported to protect hippocampal neurons *in vitro*, and additionally enhance the cognitive function of rats subjected to glutamate-mediated excitotoxicity [22].

3. Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative condition characterized by a progressive cognitive decline. The onset and progression of this disease are associated with extracellular deposits of amyloid-beta (Aβ) protein and intracellular deposits of hyperphosphorylated tau protein. Lower neurosteroid levels were observed in several brain regions of AD patients than in nondemented patients. For example, dehydroepiandrosterone sulfate (DHEAS) levels were found to be reduced in the striatum, cerebellum, and hypothalamus, and pregnenolone sulfate (PREGS) levels were reduced in the striatum and cerebellum. The levels of cortical A peptides and PREGS in the striatum and cerebellum, and the levels of phosphorylated tau and DHEAS in the hypothalamus, were negatively correlated, suggesting that these neurosteroids may affect metabolism of AD-related proteins and present neuroprotective properties [23].

Additionally, the expression of enzymes involved in the biosynthetic neurosteroid pathway was found to be upregulated in the prefrontal cortex (PFC) of AD patients. Aromatase (an enzyme that converts testosterone into estrogen) and 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1, an enzyme of the estradiol biosynthetic pathway) gene expression was upregulated in later stages of the disease. Notably, aldo-keto reductase 1C2 (AKR1C2, an enzyme of the allopregnanolone biosynthesis) gene expression was upregulated in early or mild cognitive impairment (MCI) stages of AD brains and many $GABA_A$ subunits were downregulated. The increased estradiol and allopregnanolone bioavailability may represent a compensatory neuroprotective mechanism in the PFC [24,25]. Conversely, in other study when gender was taken into consideration, allopregnanolone was found to be reduced in the PFC of male AD patients compared with age-matched controls. The authors observed an inverse correlation between allopregnanolone levels and the Braak stage, suggesting the decrease in this neurosteroid may worsen AD neuropathology [26].

3.1 Evidence of progestogens modulation of cognition and AD-related proteins in preclinical studies

AD-related proteins can modify neurosteroid production by nerve cells and *vice versa*. Wild type tau (hTau40) increased progesterone production in SH-SY5Y transfected cells, but mutant tau (P301L) did not elicit the same effect. Conversely, wild-type amyloid precursor protein (APPwt) inhibited progesterone production [27], suggesting that there are mutual and complex regulations which can affect both the neuroendocrine systems and AD-pathology. Table 1 summarizes the main preclinical findings discussed here.

Progesterone and its reduced metabolites (dihydroprogesterone and allopregnanolone) have complex effects on phosphorylation of several tau epitopes, which have been related to the modulation of kinases and phosphatases of tau protein. For example, Guerra-Araiza et al. showed that administration of progesterone and its metabolites to ovariectomized rats increased Tau-1 (dephosphorylated tau) and PHF-1 (phosphorylated tau) epitopes in the cerebellum but not in the hypothalamus. In addition, progesterone decreased GSK3β serine 9 (S9) phosphorylation, which is associated with an increase in kinase activity, suggesting that progesterone's effect on tau phosphorylation is mediated by GSK3β activation [28]. Additionally, tibolone, a synthetic steroid with progestogenic, estrogenic and androgenic activities used to treat menopausal symptoms, has been shown to reduce tau phosphorylation (PHF-1) and increase tau dephosphorylation (Tau-1) associated with GSK3β inhibition in ovariectomized rats [29].

Regarding the intracellular signaling, progesterone activity on phosphoinositide-3 kinase (PI3K)/Akt/GSK3β and mitogen-activated protein kinase (MAPK) pathways influences tau phosphorylation status and could be explored as therapeutic targets. For example, progesterone enhanced the phosphorylation of extracellular-signal regulated kinase (ERK) and Akt and increased the expression of PI3K in the cerebellar, hypothalamic and hippocampal tissues of ovariectomized rats [30]. Additionally, progesterone has been shown to stimulate the activation of tau phosphatases such as protein phosphatase 2A (PP2A) and phosphatase and tensin homolog deleted on

chromosome 10 (PTEN) in the brain, which can contribute to tau dephosphorylation [31], suggesting progesterone effects on tau phosphorylation are the result of a complex regulation of kinases and phosphatases activities.

Progesterone also displayed neuroprotective properties through several mechanisms in studies using Aβ peptides as a cellular insult. The Aβ peptide-induced neuroinflammatory response was attenuated by progesterone in cultured astrocytes through suppressing cytokine production and decreasing endoplasmic reticulum stress activation by attenuating PERK/eIF2α activity [32]. Moreover, this hormone reduced Aβ-induced cytokine production and inflammassome activation by stimulating mTOR-dependent autophagy in cultured astrocytes [33,34]. Besides inflammation, progesterone treatment attenuated Aβ-induced apoptotic mitochondrial pathway and neuronal toxicity by inactivating Jun N-terminal kinase (JNK) and activating PGRMC1 in cultured cortical neurons [35]. More recently, PGRMC1-mediated Ras signaling activation was reported to be involved in progesterone's anti-apoptotic effects in rat primary cortical neurons challenged with $A\beta_{25-35}$ [36].

Regarding CNS protection, there are complex interactions between neurosteroids. For example, estrogen, but not progesterone, exerted neuroprotective actions preventing cognitive deficits and A accumulation in ovariectomized triple transgenic AD mice (3xTg-AD). In contrast, progesterone diminished tau hyperphosphorylation alone and when in combination with estrogen, while it blocked estrogen-mediated A reduction [37]. Interestingly, progesterone alone and in combination with estrogen decreases phosphorylated abnormal tau (AT8 site) in continuous or cyclic administration, but Aβwas decreased only with cyclic progesterone administration, suggesting that estrogen and progesterone can have better results with an optimized hormone therapy while simulating physiological fluctuations [38]. Notably, progesterone and estrogen, alone or in combination, have been shown to improve spatial learning and memory performance of ovariectomized $A\beta_{1-40}$-injected rats and cognitive protection was associated with reduced neuronal apoptosis. The authors also observed cholinergic and serotonergic protection, as evidenced by increased choline acetyltransferase (ChAT) and 5-hydroxytryptamine 2A (5-HT2A) and decreased glial fibrillary acidic protein (GFAP) expression in the hippocampus. All these effects were potentiated by a combination of estrogen and progesterone [39]. Despite the growing evidence for progesterone protection in AD, there is still some debate about progesterone antagonizing estrogen's beneficial effects. Progesterone reversed the estradiol-induced spatial memory improvements in ovariectomized middle-aged rats, as evidenced by Morris water maze test scores. However, progesterone attenuated the estradiol increased mortality in these female rats [40]. Furthermore, progesterone inhibited the neuroprotective effect of 17β-estradiol against N-methyl-D-aspartate (NMDA) toxicity in hippocampal slices and reversed the estradiol-induced increase in brain derived neurotrophic factor (BDNF) protein levels and TrkB receptor activation [41].

The endogenous neurosteroid allopregnanolone has also been evaluated for its potential to promote neuroregeneration, improve cognition and reduce AD pathology [42,43]. For example, physiological concentrations of allopregnanolone (0-10 μM) prevented NMDA-mediated excitotoxicity in human NT2 neurons [44]. Corroborating these findings, the evoked presynaptic glutamate release in the rat medial prefrontal cortex was reduced by allopregnanolone by a mechanism dependent on the inhibition of L-type Ca^{2+} channels and PKA activation [45], and in the rat cortical terminal nerves, by activation of $GABA_A$ receptors. The decreased glutamate release resulted in reduced Ca^{2+} influx, suggesting that allopregnanolone may attenuate glutamate-induced excitotoxicity by a GABAergic-associated mechanism [46]. This progesterone metabolite has also been shown to restore hippocampal-dependent learning and memory associated with increased hippocampal neurogenesis in a male triple transgenic mouse model of AD (3×TgAD) [47,48]. This effect is probably related to its capacity to induce neural progenitor cells proliferation [49] and improve survival of newborn neurons [50]. In the 3×Tg-AD model, allopregnanolone reduced Aβ generation and activated microglia, increased oligodendrogenesis [50], while enhanced oligodendrocyte and neuronal differentiation [51]. These reports suggest allopregnanolone can inhibit neuroinflammation while stimulating remyelination and neurogenesis, a desirable profile for a regenerative agent for AD brain. A pharmacokinetic and pharmacodynamic study of allopregnanolone revealed that several administration routes could reach therapeutic brain and plasma concentrations resulting in increased hippocampal neurogenesis in young 3×TgAD and aged non transgenic mice [52].

Synthetic progestins have also been studied alone or in combination with estrogens in preclinical studies with regard to cognition and neuroprotection. For example, in a rat model of transitional menopause, levonorgestrel, a commonly prescribed progestin for oral contraception and menopause hormone replacement therapy (HRT), showed significant improvement in parameters such as cognition, anxiety- and depressive-like behaviors, especially when combined with estrogen. Likewise, in this study progesterone displayed better results in

combination with estrogen [53]. Conversely, levonorgestrel and estrogen combination impaired spatial working memory in middle-aged ovariectomized rats. However, both steroids were able to improve cognitive performance if administered separately [54]. In addition, the comparison of three progestins regarding cognitive performance revealed interesting neuroprotective data in middle-aged ovariectomized rats. Norethindrone acetate and medroxyprogesterone acetate (MPA) impaired working and reference memories, while levonorgestrel improved the animal learning process [55]. Such profiles may be related to their chemical structure and steroid receptor binding profile. MPA is a 17-α-hydroxyprogesterone derivative; while norethindrone acetate and levonorgestrel are 19-nortestosterone derivatives, from estrane and ethygonane groups, respectively, resembling testosterone chemical structure. Levonorgestrel displays higher affinity for testosterone and progesterone receptors compared to norethindrone acetate and MPA, whereas all of them have insignificant estrogenic activity [55]. Noteworthy, whether cognitive or neurodegenerative processes are affected by these different receptor binding affinities and chemical structures of progestins is a valid topic to be further investigated.
Moreover, progesterone and 19-norprogesterone, alone or in combination with estrogen, were able to protect hippocampal neurons against glutamate toxicity [56]. 19-norprogesterone exhibits potent progestogenic, but no glucocorticoid, estrogenic or androgenic activities [57]. In contrast, MPA did not provide this protection and reduced the estrogen-mediated benefits when administered together [56]. This was corroborated in another study, showing that MPA increased glutamate excitotoxicity in rat hippocampal neurons [58]. One hypothesis for this divergence could be attributed to BDNF gene expression and protein levels, which can be positively modulated by progesterone, while MPA did not present this profile, in cortical organotypic explants [59]. Finally, MPA treatment has also been demonstrated to impair Aβ proteolytic degradation in rat glial cells by reducing metalloproteinase 9 expression, suggesting that it may hamper Aβ degradation *in vivo* [60]. This growing evidence suggests the neuroprotective and cognitive effects depend largely on the type of progestin.

3.2 Neuronal metabolism and bioenergetics

Some lines of evidence suggest that progesterone might influence neuronal metabolism, bioenergetics and glucose uptake via non-classical pathways. For example, progesterone improved learning and memory and upregulated GLUT3, GLUT4, CREB and PPARγ in the cerebral cortex of APP/PS1 mice and primary cortical neuron cultures. The increase in neuronal glucose uptake was due to activation of the PGRMC1/CREB/GLUT3 and PGRMC1/PPARγ/GLUT4 pathways [61]. Interestingly, progesterone and other neurosteroids exhibited beneficial effects in the bioenergetic deficits of AD cell models overexpressing APP/Aβ, wtTau and the mutant Tau P301L. All of the neurosteroids tested improved mitochondrial membrane potential and ATP production in APP/Aβ cells. In contrast, only progesterone and estradiol increased ATP levels in tau P301L cells [62]. Indeed, brain mitochondria treated with estrogen or progesterone exhibit improved respiratory function through the upregulation of complex IV (cytochrome *c* oxidase) expression and attenuated oxidative stress. Hence, this could be the mechanism involved in the enhanced mitochondrial bioenergetics promoted by progesterone [63]. In addition, allopregnanolone has also been shown to improve bioenergetics in the female 3×TgAD model improving mitochondrial respiration, biogenesis and enzymes activity, together with decreased lipid peroxidation and AD pathology genes expression [64]. Moreover, pretreatment with allopregnanolone mitigated $A\beta_{25-35}$-induced neuronal death in PC12 cells and attenuated oxidative stress markers [65].
Metabolic analyses revealed that estrogen or progesterone alone improved mitochondrial respiratory capacity of toxin-challenged hippocampal neurons. However, co-administration of these two steroids decreased mitochondrial respiration. Additionally, estrogen prevented toxin-cell death, and progesterone did not, suggesting that combined therapy may not improve the mitochondrial deficits associated with AD [66]. These observations may be because progesterone largely antagonizes the estrogen-induced gene expression responses. In the brains from ovariectomized AD female macaques, genes related to mitochondrial function were upregulated by estrogen and downregulated by progesterone. While these genes upregulated by estrogen in macaques were found to be downregulated in post-mortem brains of AD female patients. This wide genomic screening indicates that estrogen decrease during menopause contributes to increased AD risk in women [67]. Therefore, experiments comparing continuous and cyclic progesterone combined with estrogen administration are necessary to help to clarify these questions and unravel their neural interactions [38].

3.3 Progestogens in epidemiological studies and clinical trials

Some evidence suggests that the age-related decrease in neurosteroids production is a crucial contributing factor to AD pathogenesis [3]. However, evidence from epidemiological studies and clinical trials are still controversial,

probably due to the divergence on the time of HRT initiation, the total time exposure to hormones and the type of progestin used, which can influence the cognitive outcomes and AD risk. Table 2 summarizes the main clinical reports discussed here. For example, a randomized double-blind controlled trial administering sequential 17-estradiol (2 mg/day) and oral natural progesterone (100 mg/day) in early postmenopausal women revealed improvements in the prefrontal cortex cognitive activity [68]. Postmenopausal women who received combined estrogen and progestin as an HRT had higher spatial cognitive test scores than the estrogen alone group. Also, serum testosterone levels were positively associated with spatial memory scores [69]. Postmenopausal women diagnosed with MCI benefited from long-term (24 months) percutaneous estrogen (2 mg/day) plus oral micronized progesterone (100 mg/day) treatment displaying better cognitive scores than the placebo group [70]. When evaluating daily life activities, a randomized prospective study reported improved scores for AD women taking conjugated equine estrogen (0.625 mg/day) plus micronized progesterone (100 mg/day) compared to tacrine. However, this HRT exhibited an equivalent efficacy on mood and cognition compared to tacrine. In addition, tacrine showed better outcomes in APOE ε4-negative patients [71].

In a retrospective case-control study, it was reported that the number of pregnancies was positively correlated with the risk of AD development, and with earlier disease onset. The AD affected group had more pregnancies than the control group, and the authors correlates the AD group risk with lifetime higher levels exposure to natural estrogen and progesterone [72]. Additionally, case-control studies reported differences regarding HRT time exposure and AD risk. On the other hand, a prospective cohort study showed that postmenopausal hormone therapy was not associated with an overall cognitive improvement in aged women. The authors also reported an increase in the risk of cognitive decline in long-term users of estrogen plus progestin or estrogen alone, and the risk increased further in women who initiated the replacement at older ages [73]. Similarly, a pilot cohort study revealed better cognitive test performance in women with early HRT initiation around menopause than individuals who initiated therapy later or were never treated. HRT was defined as with estrogen alone or estrogen plus progestin. Therefore, the timing of HRT initiation has a critical window of therapeutic opportunity [74] with a clear indication of better outcomes with early HRT initiation.

Corroborating these findings, the population-based prospective Cache County Study investigated and identified relationships between the timing of use of HRT and AD risk. When any HRT is initiated within five years after menopause begins, AD risk is reduced, especially for more than ten years of use. On contrast, AD risk was not modified in individuals starting therapy more than five years after menopause [75], further suggesting a therapeutic window for HRT. Moreover, postmenopausal women undergoing HRT with estrogen or progestogen alone or combined for up to ten years exhibited a slight increase in AD risk. However, those with more than ten years of HRT with estrogen alone had decreased AD risk. On the other hand, exposure to progestogen alone or combined did not modify the risk for AD development. This study suggests that HRT is not a determinant in AD risk and should not be indicated as a preventative strategy for cognitive impairment and dementia [76]. Another case-control study of HRT in postmenopausal women also did not detect protective effects against AD development. In a comprehensive study conducted in Finland between 1999 and 2013, women treated with any HRT presented a slightly increased risk to develop AD compared to non-treated women, independent of the age of therapy initiation and type of progestogen used [77].

Studies evaluating specific progestins were also conducted. For example, a randomized placebo-controlled clinical trial of postmenopausal women over 65 years-old treated with conjugated equine estrogen (0.625 mg/day) and the progestin MPA (2.5 mg/day) concluded that HRT increased the risk of dementia and did not protect the women from MCI development [78]. Similarly, another clinical trial evaluating conjugated equine estrogen alone (0.625 mg/day) or combined with MPA (2.5 mg/day) revealed that estrogen alone did not reduce incidence of MCI or dementia and, when the results of both HRT were pooled, it was observed an increased in the risk for both outcomes. Therefore, the authors did not recommend HRT as a preventive strategy for cognitive impairment or dementia in aged women [79]. Finally a systematic review concluded that the use of conjugated equine estrogen plus MPA was associated with increased risk of AD development [80].

Currently, two completed clinical trials in AD prevention assessing estrogen plus progesterone (NCT00000176 and NCT00006399), one trial with MPA and estrogen (NCT00066157) have been conducted with no published results with regard to progesterone or progestins and another trial with postmenopausal women using HRT including progesterone for AD neuroprotection (NCT04312399) is recruiting. A phase 1b/2a clinical trial (NCT02221622) evaluating intravenous single and multiple ascending doses for allopregnanolone (2 to 18 mg) in MCI due to AD or mild AD has been completed with promising results with regard to safety and tolerability [81]. Additionally, a phase 2 trial (NCT04838301) evaluating intravenous allopregnanolone in mild AD and another phase 1 trial

(NCT03748303) testing intramuscular allopregnanolone as a regenerative agent for early AD are not yet recruiting. Noteworthy, allopregnanolone is an approved medication for postpartum depression due to its GABAergic modulation of hypothalamic-pituitary axis [82].
Taken together, although some controversial reports are found, epidemiological and clinical trials on HRT indicate that early HRT initiation (around beginning of menopause), and natural progesterone administered alone or in combination with estrogen could result in favorable cognitive outcomes and reduce risk of AD. In contrast, some synthetic progestins such as MPA may not share this beneficial profile. Therefore, they should be further evaluated and compared for their efficacy in clinical trials and epidemiological studies.

4. Parkinson's disease

Parkinson's disease (PD) is a movement disorder characterized by tremor, rigidity and bradykinesia. Due to the loss of dopaminergic neurons in the Substantia nigra pars compacta (SNpc), reduced dopamine in striatum and intracellular α-synuclein inclusions, PD is classified as a neurodegenerative disorder. Several *in vitro* and *in vivo* studies support a potential neuroprotective role of progestogens in PD. Indeed, reports show alterations in neurosteroids levels and their synthetic enzymes in PD patients.
For example, the levels of allopregnanolone and 5α-dihydroprogesterone (5α-DHP), but not progesterone, were found to be reduced in the cerebrospinal fluid (CSF) and plasma of PD patients [83]. A parallel study with post-mortem PD brains presented reduced mRNA levels of key neurosteroid biosynthesis enzymes, neurosteroid-modulated $GABA_A$ receptor subunits and hormone receptors in the substantia nigra and striatum [84]. These results have led to the proposal that PD patients might present reduced *de novo* neurosteroid biosynthesis [85]. The results of the human studies have been corroborated in animal models [86,87]. Significant alterations were observed in neurosteroid progestogens in the brains of 6-hydroxydopamine (6-OHDA)-lesioned animals. Moreover, reduced levels of dihydroprogesterone were detected in the striatum and cerebral cortex, and pregnenolone was reduced in the striatum. Notably, isopregnanolone was increased in both brain regions. These data indicate that progesterone metabolism is compromised in the 6-OHDA PD model [88].
Previous studies demonstrated that progestogens protect dopaminergic neurotransmission in PD animal models. More specifically, in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-injected mice, progesterone and 17β-estradiol prevented striatal depletion of dopamine and its metabolites [89] and prevented dopamine transporter (DAT) downregulation in the striatum and substantia nigra [90]. Interestingly, progesterone administered after an MPTP insult also avoided reductions in dopaminergic parameters [91,92]. Progesterone increases striatal dopamine, DAT, vesicular monoamine transporter 2 (VMAT2) and BDNF levels and reduces GFAP expression in the striatum of MPTP-injected mice [92]. It has been proposed that progesterone is a potential disease-modifying treatment for the prodromal phase of PD [92]. Additionally, progesterone has neuroprotective and immunomodulatory properties in the myoenteric plexus of MPTP-lesioned mice. Progesterone has been shown to prevent MPTP-induced decreases in dopamine neurons and BDNF levels and increases in GFAP and pro-inflammatory gut macrophages [93]. This suggests progesterone can protect gut myoenteric plexus and prevent gastrointestinal alterations in PD [93]. Progesterone elicited neuroprotective and neuromodulatory effects on striatal dopaminergic, glutamatergic, and GABAergic neurotransmission systems in 6-OHDA unilaterally injected rats [94]. In addition, progesterone avoided the methamphetamine-induced striatal dopamine and serotonin depletion in ovariectomized mice [95], and significantly attenuated methamphetamine-induced striatal dopamine depletion in gonadectomized male mice [96]. In a primate model of ovariectomy, progesterone and estrogen replacement, alone or combined, improved the tyrosine hydroxylase (TH, enzyme of dopamine biossynthesis) immunoreactivity in the striatum, suggesting both hormones can protect dopamine neurons [97]. This data is corroborated by the finding that progesterone increases the number of TH-positive cells in embryonic stem cells during differentiation [98]. Together, these preclinical data suggest an important role for progesterone in dopaminergic pathway neuroprotection. Despite several reports about the beneficial effects of progesterone in preclinical PD models, controversial findings have been published. For example, chronic progesterone administration exacerbated motor impairments, and dopamine turnover in the striatum of 6-OHDA unilaterally lesioned male rats [99]. This result suggests possible adverse outcomes for male PD patients taking progesterone. A meta-analysis relating environmental and familial factors with early non-motor characteristics found no significant alteration in risk of PD associated with HRT or oral contraceptive users [100]. A case-control study observed that conjugated estrogen alone (most prescribed dose was 0.625 mg/day) increases the risk of PD in post-menopausal women with hysterectomy. However, no alteration in PD risk was detected with estrogen plus progestin (MPA) in women with natural menopause. The most prescribed dose for estrogen was 0.625 mg/day

ranging from 0.3 to 1.25 mg and for MPA 5 mg/day ranging from 2.5 to 10 mg [101]. Conversely, a conjugated equine estrogen (0.625 mg/day) followed by MPA (10 mg/day) administration reduced levodopa-induced dyskinesia in post-menopausal PD patients [102]. Another case-control study found that esterified estrogen administered alone or in combination with a progestin increased PD risk. The same study also showed that conjugated estrogen alone or combined with progestin did not modify PD risk [103], suggesting that the conjugated form of estrogen maybe be safer. Moreover, a clinical trial evaluating motor function impairment in PD patients revealed an anti-dopaminergic effect for progesterone treatment (100 mg/day), whereas 17β-estradiol administration (2 mg/day) presented no effect [104]. Despite several preclinical evidence of progesterone improvements in PD models, these findings are not supported by epidemiological studies that show no alterations or even increase in PD risk for progestins combined with estrogen, and further epidemiological and mechanistical studies are needed to elucidate this topic. Currently, there are no studies registered on clinicaltrials.gov assessing progestins for PD.

Allopregnanolone as a neuroprotective agent in PD is a relatively new area of research. The ipsilateral injection of allopregnanolone in 6-OHDA hemiparkinsonian rats improved the contralateral rotational behavior, considered a sign of motor degeneration [105]. Moreover, early progesterone treatment enhanced the ipsilateral activity and expression of 3α-hydroxysteroid oxidoreductase, which is involved in converting progesterone to allopregnanolone. Thus, some progesterone-mediated neuroprotective effects could actually be mediated by allopregnanolone [105]. Chronic post-lesion allopregnanolone treatment improved the cognitive deficits in spatial and recognition memories in 6-OHDA-lesioned male rats [106]. Allopregnanolone also protected 6-OHDA-injured SH-SY5Y cells resulting in increased tyrosine-hydroxylase expression. This effect was dependent on activation of $GABA_A$ receptors and modulation of Ca^{2+}/calmodulin (CaM)-dependent protein kinase II δ3 (CaMKIIδ3)/BDNF signaling pathway [107]. Currently, to our knowledge, there are no epidemiological studies or ongoing clinical trials evaluating allopregnanolone potential to halt PD progression.

5. Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disease caused by a polyglutamine repeat in the huntingtin gene, ultimately transcribed and translated into mutant huntingtin (mHtt) protein. This disease leads to a progressive loss of striatal GABAergic neurons, causing cognitive, neuropsychiatric and motor impairments such as tremor, chorea and dystonia. Both genders have equal prevalence because of its inherited autosomal dominant pattern, but neurosteroids such as estrogens have provided neuroprotection in experimental models [86,108].

The mitochondrial complex II inhibitor, 3-nitropropionic acid, produces selective striatal lesions and has been systemically administered in rodents to model HD. In this model, chronic treatment with progesterone improved motor performance and antioxidant enzymes and attenuated oxidative stress and inflammatory cytokines. In contrast, pretreatment with pregnenolone, a $GABA_A$ receptor negative modulator, reversed the beneficial progesterone-mediated effects [109]. This observation suggests that progesterone's protective effects might be mediated by its metabolite allopregnanolone, a $GABA_A$ receptor positive modulator. Additionally, the neurosteroids allopregnanolone and progesterone reduced mHtt aggregates in cultured astrocytes by inducing mTOR-dependent autophagy [110]. Progestogens are relatively unexplored compounds for HD with a few preclinical studies published, and currently, to our knowledge, there are no ongoing clinical trials evaluating progesterone or allopregnanolone neuroprotection to modify HD course.

6. Amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder also known as motoneuron disease or Lou-Gehrig's disease. It causes the progressive death of motoneurons from brain stem and spinal cord, leading to several symptoms such muscle spasms, weakness and atrophy, pain, cognitive and emotional alterations, and respiratory failure. The later is commonly the cause of death between, 3-5 years from final diagnosis [111,112]. Some lines of evidence point out a possible protective role for progesterone in ALS. It has been demonstrated elevated progesterone serum levels in ALS patients compared to controls and a positive correlation with better prognosis such as survival time; whereas this protective profile was not shared by cortisol [113,114]. Additionally, the classical progesterone receptors PR-A and B were found to be increased in the human spinal cord in ALS, especially in the lumbar and cervical parts, in axons and blood vessels, with their immunoreactivity increased in nerve roots and large arteries [115]. This might suggest a protective role for progesterone in an attempt to repair tissue degeneration.

Genetic mouse models have been developed to partially resemble some ALS features. For example, the Wobbler mice show spontaneous spinal cord and brain stem motoneuron degeneration and astrogliosis [112]. Similarly to the human findings, increased progesterone, allopregnanolone and 20⍰-dihydroprogesterone levels were found in the brain, spinal cord and also in the adrenal glands of Wobbler mice [116]. Progestogens have exhibited beneficial effects in ALS models characterized by spinal cord motor neuron degeneration. In the brain of these animals, progesterone increased GABAergic interneurons and granule cells, along with decrease in astrocytes number in the hippocampus; however, no influence on neurogenesis was observed by the authors [117]. Treatment of Wobbler mice with progesterone attenuated neuropathy, improved motor neuron morphology and restored Na^+,K^+-ATPase pump mRNA levels [118]. ChAT immunoreactivity was also increased in motoneurons after progesterone administration [119,120]. The beneficial effects on Wobbler mice neuropathology might be related to increased BDNF mRNA levels both in spinal cord motoneurons [119,121] and in the hippocampus [117]. Glial cells are also positively influenced by progestagens. For instance, GFAP-positive astrocytes were decreased by progesterone in the spinal cord motoneurons [120]. The oligodendrocyte density in the spinal cord gray matter of Wobbler mice were increased by progesterone treatment [121], indicating its remyelinating potential (see Multiple sclerosis session). In the same model, progesterone reversed the pro-inflammatory macroglial phenotype and inflammatory mediators, together with enhanced ChAT expression, effects counteracted by the synthetic progestin norethindrone. This progestin is a 19-nor-testosterone derivative exhibiting estrogenic and androgenic activities besides the PR activation which may lead to unwanted outcomes [122]. These results indicate that the type of progestin used may produce different results probably related to the hormone steroid receptor profile activation. Progesterone and allopregnanolone also have been shown to improve several parameters in Wobbler mice. These hormones improved neuronal vacuolation, nitric oxide synthase (NOS) hyperactivity and cell survival markers after acute treatment. Also, both molecules improved manganese superoxide dismutase (MnSOD) immunoreactivity, BDNF mRNA levels and muscle performance after chronic treatment [123]. In another transgenic mouse model of ALS expressing a mutant human superoxide dismutase 1 (G93A-SOD1), progesterone reduced spinal cord motor neuron death, delayed motor neuron dysfunction progression and increased animal lifespan. It was demonstrated that these effects were associated with autophagic flux activation and downregulation of the mutant SOD1 expression [124].
Other synthetic progestins such as Nestorone have been tested for alleviating ALS symptoms and neuropathology [111]. Interestingly, Nestorone-treated Wobbler mice showed improvement in several spinal cord abnormalities, i.e., restored ChAT-IR, decreased motoneuron vacuolization, astrogliosis and microgliosis, and down-regulated proinflammatory markers. In addition, a slight enhancement in forepaw anatomy was observed after Nestorone, supporting this progesterone receptor agonist as a promising strategy for ALS [125]. Nestorone, a 19-norprogesterone derivative, has a unique profile among progestins with stronger progestogenic activity than progesterone itself due to its high affinity for PR and very low activities at androgen, estrogen and glucocorticoid receptors [126].
Currently there are no studies on clinicaltrials.gov evaluating progestogens repurposing for ALS. Nonetheless, epidemiological studies were conducted to assess a possible relationship between menopause, HRT and ALS development. A case-control study found no association between several reproductive factors (such as age at menarche and final menstrual period, type of menopause, and others) and ALS risk. Additionally, HRT during post-menopause showed a slight but insignificant association with ALS risk [127]. A large case-control study revealed no significant association between estrogen and progestogen exposure and a possible reduced ALS risk. Notably, among the three countries assessed, HRT was only associated with decreased ALS risk in the Netherlands [128].

7. Multiple sclerosis

Another important aspect of progesterone therapeutic profile is its reported role in myelin formation and remyelinating processes. In this sense, the use of natural progesterone or synthetic progestins is considered an attractive therapeutic approach for treating demyelinating diseases, such as multiple sclerosis (MS) or inducing myelin repair after injuries [129]. It was observed that levels of neurosteroids, particularly progesterone and testosterone metabolites, are altered in the CSF and plasma of male MS patients [130]. For a review on the levels and sex differences of steroid hormones in neurodegenerative diseases see Giatti et al. [131].
In a mouse model of sciatic nerve injury, it was observed that progesterone is generated from pregnenolone by Schwann cells in peripheral nerves, and an increase in progesterone or pregnenolone levels augment myelin sheath formation rate. A positive progesterone effect on axon myelination was also demonstrated in rat dorsal root ganglia *in vitro* [132]. These benefits are suggested to be related to progesterone actions in

oligodendrogenesis and myelin formation in the CNS and PNS [129]. Progesterone increased the proliferation rate of oligodendrocyte precursors cells (OPC) via classical PR activation in organotypic slice cultures of the rat cerebellum [133], stimulated the differentiation of OPCs into mature myelinating oligodendrocytes [133] and upregulated the gene expression of myelin basic protein (MBP), a myelin sheath component, a process that is dependent on PR activation [134]. Allopregnanolone could also increase MBP expression in a $GABA_A$ receptor-dependent manner [134].

In a spinal cord injury rat model, progesterone treatment after lesion increased OPC proliferation and upregulated the mRNA levels of transcription factors Olig2 and Nkx2.2, which are required for oligodendrocyte differentiation, and later Olig1, which is involved in myelin repair. Progesterone also increased the mRNA and protein levels of MBP and proteolipid protein (PLP). These effects resulted in increased mature oligodendrocytes and remyelination [135]. Besides stimulating oligodendrocyte differentiation and maturation, progesterone reduces astrocyte and microglia proliferation and activation [136].

In a demyelination mouse model, the effects of progesterone on microglial cells are reported to involve a switch from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype and NLRP3 inflammasome suppression [137]. Similar effects were observed in an MS model induced by autoimmune encephalomyelitis. In this model, progesterone reduced inflammatory cell infiltration in the injured spinal cord, prevented demyelination and attenuated disease severity [138]. Therefore, the evidence suggests that progesterone can act as a remyelinating and anti-inflammatory agent, suppressing reactive gliosis in MS.

There is also evidence from clinical studies on the possible benefits of treating MS patients with progesterone. In a retrospective pilot study, women with MS were questioned about the disease severity. The majority (82%) of the premenopausal women reported worse symptoms during the premenstrual period, 54% of post-menopausal women reported increased disease severity after menopause, and 75% of HRT users reported an enhancement in symptoms severity [139]. Conversely, a systematic review with menopausal MS patients reported an inconclusive association between age at menopause, use of HRT and disease severity [140]. Still, a cohort observational study revealed that HRT (at least one year of systemic estrogen with or without a progestin) in the post-menopausal phase resulted in a better physical quality of life in women with MS [141]. In a randomized controlled clinical trial (NTC00127075), the administration of nomegestrol acetate (a 19-norprogesterone derivative, 10 mg/day) and transdermal estradiol (75 µg/week) to MS women in the post-partum phase reduced disease relapse [142].

8. Conclusions and future perspectives

This review summarized preclinical and clinical studies addressing the progestogen potential for some of the most common neurodegenerative diseases: AD, PD, HD, ALS and MS. There are still many challenges from basic experimental science to clinical translation for effective HRT in neuroprotection. For example, evidences from animal models demonstrate that cyclic administration of progesterone and estrogen resembling the natural menstrual cycle yields better results than continuous treatment [38]. Also, the benefits of HRT seem to be higher with early initiation around menopause rather than late usage [73–75]. Thus, estrogen-combined and cyclic treatment of progestogens in the early menopause might provide important results in clinical trials for neurodegenerative disorders. Some studies reveal a promising profile for natural progesterone with regard to AD risk and progression than synthetic progestins, especially MPA, which appears to increase the risk [75,78,80]. Nestorone shows a promising profile for ALS in preclinical studies [111,125]. In addition, the progesterone metabolite allopregnanolone showed very promising results in preclinical studies and the ongoing and future trials may confirm its regenerative potential.

In summary, despite reports of non-effectiveness or reversion of beneficial estrogen-related effects, preventive and therapeutic use of progesterone, allopregnanolone and synthetic progestins are important to be considered in future studies, regarding the mechanism of action, once it may modify the course of some neurodegenerative conditions. In this sense, dose, administration regimen, timing, the involved complex intracellular signaling pathways and the potential benefits of post-menopausal progestogen therapy need to be more thoroughly evaluated, especially when administered in combination with estrogen.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Author contributions

Taysa Bervian Bassani drafted the work, performed the research and interpretation of data. Taysa Bervian Bassani and Rodrigo Portes Ureshino concepted the idea, revised and corrected the final version. Cynthia Silva Bartolomeo and Rafaela Brito Oliveira draw the figures and revised the manuscript. All authors approved the final version of this manuscript.

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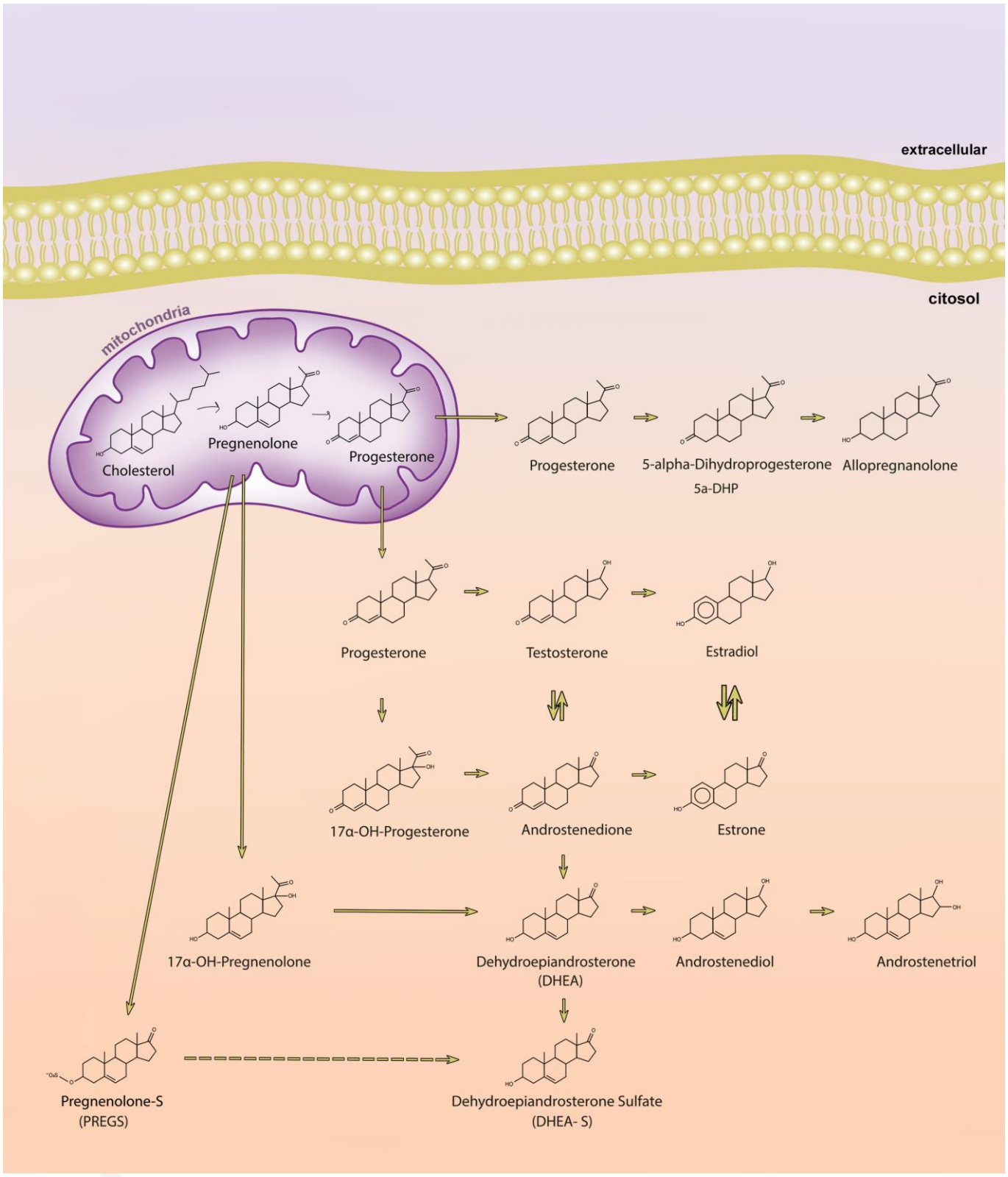
Figure captions

Figure 1: Steroid metabolism in the Central Nervous System. Neurosteroids are biosynthesized in the Central Nervous System (CNS) and Peripheral Nervous System (PNS) by neuronal and glial cells from cholesterol. Neuroactive steroids can also be synthesized peripherally by adrenal and gonadal glands, cross the blood-brain barrier and act on steroid receptors in the CNS. The first limiting step in steroid synthesis is the conversion of cholesterol to pregnenolone.

Figure 2: Mechanisms of action of progesterone and allopregnanolone on the Central Nervous System. Two main pathways mediate the effects of progesterone on target cells: the classical (canonic pathway) and non-classical (non-canonic pathway). In the classical signaling pathway, both progesterone and 5α-dihydroprogesterone (5α-DHP) bind to intracellular progesterone receptors (PR), which dimerize and translocate to the nucleus, thus interacting with the regulatory progesterone response elements (PRE) in the DNA, which regulates the expression of specific genes. While in the non-classical pathway, there is activation of G protein-coupled membrane

progesterone receptors (mPRs) and progesterone receptor membrane component 1 (PGRMC1), leading to activation of MAPK, protein kinase C (PKC), and PI3K / Akt pathways. 5α-dihydroprogesterone is converted to allopregnanolone. The latter has no affinity for PR but is a positive modulator of type A γ-aminobutyric acid receptors ($GABA_A$) and is also a ligand for mPRs. MBP: myelin basic protein, ΔΨm: mitochondrial membrane potential.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Estradiol
17α-OH-Progesterone
Androstenedione
Estrone
17α-OH-Pregnenolone
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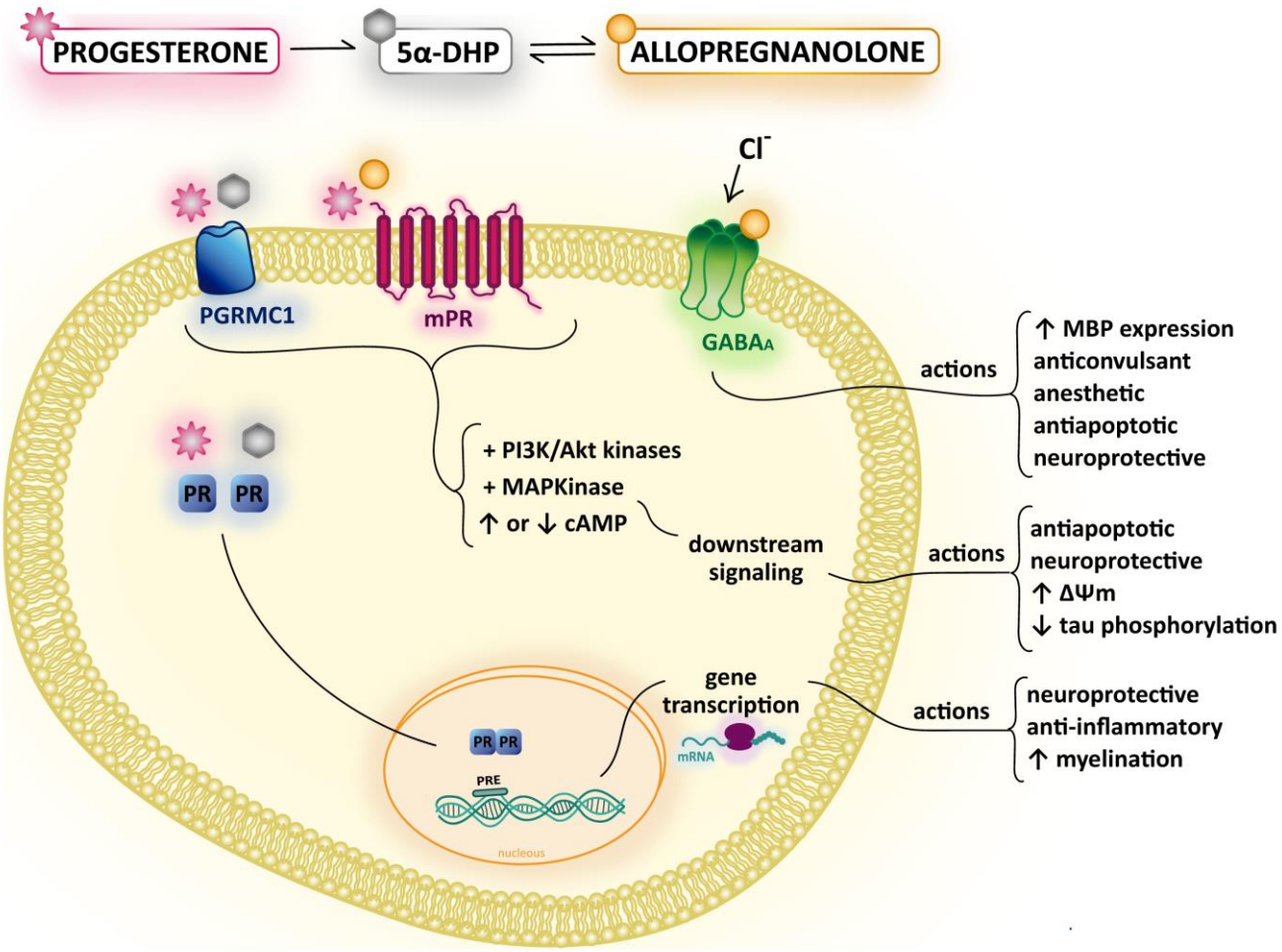
PROGESTERONE
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antiapoptotic
neuroprotective
+ PI3K/Akt kinases
+ MAPKinase
↑ or ↓ cAMP
downstream signaling
actions
antiapoptotic
neuroprotective
↑ ΔΨm
↓ tau phosphorylation
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PR
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neuroprotective
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Table 1. Evidence of progestogens effects in preclinical models of neurodegenerative diseases

Hormones	Model	Main outcomes	Reference
Progesterone	SH-SY5Y transfected cells	Wild type tau (hTau40) increased progesterone production, but not mutant tau (P301L); wild-type amyloid precursor protein (APPwt) inhibited progesterone production	27
Progesterone and its reduced metabolites	Ovariectomized rats	Increased Tau-1 (dephosphorylated tau) and PHF-1 (phosphorylated tau) epitopes in the cerebellum, but not in the hypothalamus; increased GSK3β activation	28
Tibolone	Ovariectomized rats	Increased tau dephosphorylation (Tau-1) and reduced tau phosphorylation (PHF-1) associated with GSK3β inhibition	29
Progesterone	Ovariectomized rats	Enhanced phosphorylation of ERK and Akt and increased expression of PI3K in the cerebellar, hypothalamic and hippocampal tissues	30
Progesterone	Ovariectomized rats	Increased activation of the tau phosphatases such as PP2A and PTEN in the brain	31
Progesterone	Astrocytes cell culture	Attenuated Aβ peptide-induced neuroinflammation; suppressed cytokine production and decreased endoplasmic reticulum stress activation by attenuating PERK/eIF2α	32
Progesterone	Astrocytes cell culture	Attenuated Aβ-induced cytokine production and inflammassome activation by stimulating mTOR-dependent autophagy	33,34
Progesterone	Cortical neurons culture	Reduced Aβ-induced apoptotic mitochondrial pathway and neuronal toxicity by inactivating JNK and activating PGRMC1	35
Progesterone	Rat primary cortical neurons challenged with $A\beta_{25-35}$	Anti-apoptotic effects mediated by PGRMC1 activation of Ras signaling	36
Progesterone	Ovariectomized triple transgenic AD mice (3xTg-AD)	Diminished tau hyperphosphorylation alone and combined with estrogen, but blocked estrogen-mediated Aβ reduction	37
Progesterone	Female 3xTransgenic-AD mice	Decreases phosphorylated abnormal tau (AT8 site) in continuous or cyclic administration, but Aβ was decreased only with cyclic progesterone	38
Progesterone	Ovariectomized $A\beta_{1-40}$-injected rats	Improved spatial learning and memory; reduced neuronal apoptosis; cholinergic and serotonergic protection; decreased GFAP expression in the hippocampus; effects were potentiated by a combination of estrogen and progesterone	39
Progesterone	Ovariectomized middle-aged rats	Reversion of estradiol-induced spatial memory improvements, but attenuation of the estradiol increased mortality	40
Progesterone	Rat hippocampal slices challenged with NMDA	Inhibition of 17β-estradiol neuroprotective effect; reversion in the estradiol-induced increase in BDNF protein levels and TrkB receptor activation	41
Allopregnanolone	Human NT2 neurons	Prevention of NMDA-mediated excitotoxicity	44
Allopregnanolone	Organotypic slices culture of rat medial prefrontal cortex	Reduced evoked presynaptic glutamate release dependent on inhibition of L-type Ca^{2+} channels and PKA activation	45
Allopregnanolone	Rat cortical terminal nerves (synaptosomes)	Decreased glutamate release and Ca^{2+} influx by activation of $GABA_A$ receptors	46
Allopregnanolone	Male 3x transgenic mouse model of AD (3×TgAD)	Restored hippocampal-dependent learning and memory associated with increased hippocampal neurogenesis	47,48
Allopregnanolone	Rat and human neural progenitor cells	Induced neural progenitor cells proliferation	49
Allopregnanolone	3×Tg-AD model	Improved survival of newly generated neurons; reduced Aβ generation and activated microglia, increased oligodendrogenesis	50
Allopregnanolone	Adult mouse neural stem cells and 3×Tg-AD model	Enhanced oligodendrocyte and neuronal differentiation	51
Allopregnanolone	Young 3×TgAD and aged non transgenic mice	Increased hippocampal neurogenesis	52
Levonorgestrel	Rat model of transitional menopause	Improvement in cognition, anxiety- and depressive-like behaviors, especially when combined with estrogen.	53
Levonorgestrel	Middle-aged ovariectomized rats	Impaired spatial working memory with estrogen combination, but improved cognitive performance if administered separately	54
Norethindrone acetate, MPA, Levonorgestrel	Middle-aged ovariectomized rats	Norethindrone acetate and medroxyprogesterone impaired working and reference memories; levonorgestrel improved the learning process	55
Progesterone, 19-norprogesterone and MPA	Rat hippocampal neurons challenged with glutamate	Progesterone and 19-norprogesterone promoted neuronal protection, but not MPA and it reduced the estrogen-mediated benefits when administered together; MPA increased glutamate excitotoxicity	56, 58
Progesterone and MPA	Cortical organotypic explants	Progesterone increased BDNF gene expression and protein levels, but not MPA	59
MPA	Rat glial cells	Impaired Aβ proteolytic degradation by reducing metalloproteinase 9 expression	60
Progesterone	Cerebral cortex of APP/PS1 mice and primary cortical neuron cultures	Improved learning and memory, upregulated GLUT3, GLUT4, CREB, PPARγ, and increased neuronal glucose uptake through PGRMC1 activation	61
Progesterone and 17β-estradiol	APP/Aβ and tau P301L cells	Improved mitochondrial membrane potential and ATP production	62

Progesterone and 17β-estradiol	brain mitochondria	Improved respiratory function through the upregulation of complex IV (cytochrome *c* oxidase) expression and attenuated oxidative stress	63
Allopregnanolone	Female 3×TgAD model	Improved mitochondrial respiration, biogenesis and enzymes activity, decreased lipid peroxidation and expression of AD-related genes	64
Allopregnanolone	$A\beta_{25\text{-}35\text{-}}$ challenged PC12 cells	Attenuated neuronal death and oxidative stress markers	65
Progesterone and 17β-estradiol	Toxin-challenged hippocampal neurons	Estrogen or progesterone alone improved mitochondrial respiratory capacity; co-administration decreased mitochondrial respiration; estrogen prevented cell death, and progesterone did not	66
Progesterone and estrogen	Ovariectomized Rhesus macaques; female AD patients and controls	Genes related to mitochondrial function were upregulated by estrogen and downregulated by progesterone in macaques brains; the genes upregulated by estrogen in macaques were downregulated in post-mortem brains of AD patients	67
Progesterone and 17β-estradiol	MPTP-injected mice	prevented striatal depletion of dopamine and its metabolites, and prevented dopamine transporter (DAT) downregulation in the striatum and substantia nigra	89, 90
Progesterone	MPTP-injected mice	Increased striatal dopamine, DAT, vesicular monoamine transporter 2 (VMAT2) and BDNF levels and reduced GFAP expression in the striatum	91, 92
Progesterone	MPTP-injected mice	Prevented the decrease in dopamine neurons and BDNF levels and the increase in GFAP and pro-inflammatory gut macrophages, protecting myoenteric plexus	93
Progesterone	6-OHDA unilaterally injected rats	Neuroprotective and neuromodulatory effects on striatal dopaminergic, glutamatergic, and GABAergic neurotransmission systems	94
Progesterone	Methamphetamine-injected ovariectomized mice	Prevented the striatal dopamine and serotonin depletion	95
Progesterone	Methamphetamine-injected gonadectomized male mice	Attenuated striatal dopamine depletion	96
Progesterone and estrogen	Primate model of ovariectomy	Improved the TH-IR in the striatum and protected dopamine neurons	97
Progesterone	Embryonic stem cells	Increased the number of TH-positive cells during differentiation	98
Progesterone	6-OHDA unilaterally lesioned male rats	Chronic administration exacerbated motor impairments, and dopamine turnover in the striatum	99
Allopregnanolone	6-OHDA hemiparkinsonian rats	Improved the contralateral rotational behavior, a sign of motor degeneration	105
Allopregnanolone	6-OHDA-lesioned male rats	Chronic post-lesion treatment improved the cognitive deficits in spatial and recognition memories	106
Allopregnanolone	6-OHDA-injured SH-SY5Y cells	Increased TH expression dependent on activation of $GABA_A$ receptors and modulation of CaMKIIδ3/BDNF signaling pathway	107
Progesterone and pregnenolone	3-nitropropionic acid-injected rats model of Huntington's disease	Progesterone improved motor performance, antioxidant enzymes and attenuated oxidative stress and inflammatory cytokines; pregnenolone reversed these effects	109
Allopregnanolone and progesterone	Cultured astrocytes	Reduced mutant huntingtin (mHtt) aggregates by inducing mTOR-dependent autophagy	110
Progesterone	Wobbler mice model of ALS	Increased GABAergic interneurons and granule cells, decreased astrocyte number and increased *BDNF* mRNA levels in the hippocampus; no influence on neurogenesis	117
Progesterone	Wobbler mice model of ALS	improved motor neuron morphology and Na^+,K^+-ATPase sodium pump mRNA levels; attenuated neuropathy	118
Progesterone	Wobbler mice model of ALS	Increased ChAT-IR and BDNF mRNA levels in spinal cord motoneurons	119
Progesterone	Wobbler mice model of ALS	Decreased GFAP-positive astrocytes and increased ChAT-IR in the spinal cord motoneurons	120
Progesterone	Wobbler mice model of ALS	Increased *BDNF* mRNA levels and oligodendrocyte density in spinal cord motoneurons	121
Progesterone and norethindrone	Wobbler mice model of ALS	Progesterone reversed the pro-inflammatory macroglial phenotype and inflammatory mediators and enhanced ChAT expression; norethindrone inhibited these effects	122
Progesterone and allopregnanolone	Wobbler mice model of ALS	Acute treatment: improved neuronal vacuolation, nitric oxide synthase hyperactivity and cell survival markers. Chronic treatment: improved manganese superoxide dismutase (MnSOD)-IR, BDNF mRNA levels and muscle performance	123
Progesterone	Mutant human superoxide dismutase 1 (G93A-SOD1) transgenic mouse model of ALS	Reduced spinal cord motor neuron death, delayed motor neuron dysfunction progression and increased animal lifespan associated with autophagic flux activation and downregulation of the mutant SOD1	124
Nestorone	Wobbler mice model of ALS	Restored ChAT-IR, decreased motoneuron vacuolization, astrogliosis, microgliosis, and proinflammatory markers	125
Progesterone and pregnenolone	Mouse model of sciatic nerve injury; rat dorsal root ganglia	Increase in progesterone or pregnenolone levels enhances myelin sheath formation rate by Schwann cells in peripheral nerves; progesterone improved axon myelination in rat dorsal root ganglia *in vitro*	132
Progesterone	Organotypic slice cultures of rat cerebellum	Increased proliferation rate of OPC via classical PR activation; enhanced differentiation of OPCs into mature myelinating oligodendrocytes	133
Progesterone and allopregnanolone	Organotypic slice cultures of rat cerebellum	Upregulated gene expression of myelin basic protein (MBP) dependent on PR and $GABA_A$ receptors activation	134
Progesterone	Spinal cord injury rat model	Increased OPC proliferation; upregulated mRNA levels of transcription factors (Olig1, Olig2 and Nkx2.2) required for oligodendrocyte differentiation and myelin repair; increased mRNA and protein levels of MBP and proteolipid protein (PLP); increased mature oligodendrocytes and remyelination	135

Progesterone	Spinal cord injury rat model	Stimulation of oligodendrocyte differentiation and maturation, and late remyelination; inhibition of astrocyte and microglia proliferation and activation	136
Progesterone	Cuprizone-induced demyelination mouse model of MS	Switch from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype in microglial cells and NLRP3 inflammasome suppression	137
Progesterone	Experimental autoimmune encephalomyelitis MS model	Reduced inflammatory cell infiltration in the injured spinal cord, prevented demyelination and attenuated disease severity	138

Alzheimer's disease (AD); Amyotrophic Lateral Sclerosis (ALS); Brain derived neurotrophic factor (BDNF); Choline Acetyltransferase (ChAT); γ-Aminobutyric acid (GABA); Glial Fibrillary Acidic Protein (GFAP); 6-hydroxydopamine (6-OHDA); Medroxyprogesterone acetate (MPA); 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); Multiple Sclerosis (MS); oligodendrocyte precursors cells (OPC); Tyrosine Hydroxylase (TH); immunoreactivity (IR).

Table 2. Epidemiological studies and clinical trials associating HRT with the risk to develop neurodegenerative diseases or modify the disease course

Hormones	Subjects	Type of study	Main outcomes	Reference
17β-estradiol and oral natural progesterone	Early postmenopausal women	Randomized, double blind, placebo-controlled crossover study	Improvements in the prefrontal cortex cognitive activity	68
Estrogen alone or combined with a progestin	Postmenopausal women	Cross-sectional study	Higher spatial cognitive test scores for women currently receiving estrogen plus progestin compared to estrogen alone; testosterone levels correlated positively with higher spatial test scores	69
Long-term percutaneous estrogen plus oral micronized progesterone	Postmenopausal women diagnosed with MCI	Randomized, placebo-controlled clinical trial	Better cognitive scores and slow progression rate to dementia in the HRT group compared to placebo	70
Progesterone plus estrogen compared to tacrine	Postmenopausal AD patients	Randomized open-label, prospective clinical trial	HRT improved scores in daily life activities, but had an equivalent efficacy compared to tacrine on cognition and mood	71
Progestin plus estrogen or estrogen alone	Postmenopausal women aged 71 to 81 years	Prospective cohort study	Postmenopausal hormone therapy was not associated with an overall cognitive improvement; increase in the risk of cognitive decline in long-term users, particularly those who initiated the HRT at older ages	73
HRT with estrogen alone or combined with progestin	Menopausal and postmenopausal women	Pilot cohort study	Early HRT initiation around menopause resulted in better cognitive test performance than individuals who initiated therapy later or were never treated.	74
HRT	Postmenopausal women	Population-based prospective observational study	Reduced AD risk when HRT is initiated within five years after menopause; AD risk was not changed when therapy started more than five years after menopause	75
HRT with estrogen or progestogen alone or combined	postmenopausal women	Case-control study	HRT for up to ten years resulted in a slight increase in AD risk; more than ten years of HRT with estrogen alone decreased AD risk; exposure to progestogen alone or combined did not modify the risk for AD; HRT was not considered a determinant in AD risk	76
HRT with estrogen or progestogen alone or combined	Postmenopausal women	Nationwide case-control study	Any HRT slightly increased risk to develop AD, independent of the age of therapy initiation and type of progestogen used	77
Conjugated equine estrogen and MPA	Postmenopausal women over 65 years-old	Randomized placebo-controlled clinical trial	HRT increased the risk of dementia and did not protect the women from MCI development	78
Conjugated equine estrogen alone or combined with MPA	Postmenopausal women 65 to 79 years-old	Randomized placebo-controlled clinical trial	Estrogen alone did not reduce incidence of MCI or dementia; pooled results of estrogen alone and estrogen plus MPA showed an increase in the risk for both outcomes.	79
Conjugated equine estrogen plus MPA	-	Systematic review	Increased risk of AD development	80
HRT or oral contraceptive use	-	Meta-analysis	No significant alteration in the risk of PD development	100
Estrogen alone and estrogen plus progestin	Postmenopausal women	Case-control study	Estrogen alone increases the risk of PD in hysterectomized post-menopausal women; estrogen plus progestin did not alter PD risk in women with natural menopause	101
Conjugated equine estrogen followed by MPA	Post-menopausal women aged 45 to 75 years-old	Double-blind, placebo-controlled, crossover study	HRT reduced levodopa-induced dyskinesia in PD post-menopausal patients	102
Esterified or conjugated estrogen alone or combined with a progestin	Idiopathic PD women from 35 to 89 years-old and aged matched controls	Population-based case-control study	Increased in PD risk for esterified estrogen combined with a progestin; no alteration in PD risk for conjugated estrogen alone or combined with a progestin	103
Progesterone or 17β-estradiol	Postmenopausal PD women	Placebo-controlled randomized clinical trial	Anti-dopaminergic effect for progesterone in the motor function of PD patients; 17β-estradiol administration presented no effect	104

HRT	Post-menopausal women and controls	Case-control study	Both HRT during post-menopause and reproductive factors showed no significant association with ALS risk.	127
HRT	ALS patients and matched controls from the Netherlands, Ireland and Italy	Case-control study	No alteration in ALS risk and estrogen and progestogen exposure; only in the Netherlands HRT was associated with a decreased risk for ALS	128
HRT	Premenopausal and postmenopausal women with MS	Retrospective pilot study	HRT users reported an improvement in MS symptoms severity	139
HRT	Menopause women with MS	Systematic review	Inconclusive association between age at menopause, HRT and MS disease severity	140
HRT with at least one year of systemic estrogen with or without a progestin	Post-menopausal MS women and aged matched controls	Cohort observational study	Systemic HRT use was associated with better physical quality of life in women with MS	141
Nomegestrol acetate plus transdermal estradiol	Women in the post-partum phase	Randomized, placebo controlled double-blind clinical trial	Nomegestrol acetate (19-norprogesterone derivative) and estrogen reduced MS relapse in the post-partum phase	142

AD: Alzheimer's disease; ALS: Amyotrophic Lateral Sclerosis; HRT: Hormone Replacement Therapy; MCI: Mild Cognitive Impairment; MPA: Medroxyprogesterone acetate; MS: Multiple Sclerosis; PD: Parkinson's disease.