

REVIEW ARTICLE

Allopregnanolone: An overview on its synthesis and effects

Silvia Diviccaro | Lucia Cioffi | Eva Falvo | Silvia Giatti | Roberto Cosimo Melcangi 

Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italy

Correspondence

Roberto Cosimo Melcangi, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy, Via Balzaretti 9, 20133 Milano, Italy.
Email: roberto.melcangi@unimi.it

Funding information

MIUR Progetto Eccellenza; PON "Ricerca e Innovazione" PerMedNet, Grant/Award Number: ARS01_01226; PRIN, Grant/Award Number: 2017ZFJCS3; Intramural Grant Line-2 Action-A, Grant/Award Number: PSR2020

Abstract

Allopregnanolone, a $3\alpha,5\alpha$ -progesterone metabolite, acts as a potent allosteric modulator of the γ -aminobutyric acid type A receptor. In the present review, the synthesis of this neuroactive steroid occurring in the nervous system is discussed with respect to physiological and pathological conditions. In addition, its physiological and neuroprotective effects are also reported. Interestingly, the levels of this neuroactive steroid, as well as its effects, are sex-dimorphic, suggesting a possible gender medicine based on this neuroactive steroid for neurological disorders. However, allopregnanolone presents low bioavailability and extensive hepatic metabolism, limiting its use as a drug. Therefore, synthetic analogues or a different therapeutic strategy able to increase allopregnanolone levels have been proposed to overcome any pharmacokinetic issues.

KEYWORDS

neuroactive steroids, neurodegenerative disorders, psychiatric disorders, progesterone metabolism, sex difference

1 | INTRODUCTION

Progesterone (PROG) not only comprises a physiological regulator of reproduction,¹⁻⁵ but also exerts important effects in the nervous system. Indeed, this neuroactive steroid regulates development of neurones⁶⁻⁹ and glial cells,¹⁰⁻¹³ as well as the myelination process.¹⁴⁻¹⁸ In addition, PROG exerts important protective effects in neurodegenerative and psychiatric disorders.^{15,19-27} However, whether the effects of PROG are the result of itself and/or its metabolites is still poorly considered. Among PROG metabolites, the effects of allopregnanolone (ALLO), also known as tetrahydroprogesterone, in the nervous system have attracted the attention of several researchers. Therefore, even if many aspects of this neurosteroid remain to be clarified, an extensive literature on it is now available. In the present review, we discuss the state of art of this neuroactive steroid, considering its synthesis, mechanism of actions, and physiological and protective effects. In addition, whether neurodegenerative and psychiatric disorders, as well as peripheral steroid contents, influence

the amount of this neuroactive steroid in the nervous system and whether sex dimorphism may occur are also taken into consideration.

2 | SYNTHESIS AND MECHANISM OF ACTION

In the nervous system, PROG is actively converted by the enzyme 5α -reductase (5α -R) into dihydroprogesterone (DHP) and subsequently by the action of the enzymes 3α -hydroxysteroid oxidoreductase or 3β -hydroxysteroid oxidoreductase into ALLO and isoallopregnanolone (ie, the 3β -isomer of ALLO).^{28,29} Two isoforms of 5α -R, called type 1 and type 2, are responsible for the metabolism of neuroactive steroids, including PROG.³⁰⁻³³ Type 1 isoform is expressed in cortical, hippocampal and olfactory bulb glutamatergic neurones and in some output neurones of the amygdala and thalamus,³⁴ with high levels in midbrain, corpus callosum, anterior commissure, optic chiasm, pons and spinal cord,^{33,35,36} and particularly in purified myelin

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. *Journal of Neuroendocrinology* published by John Wiley & Sons Ltd on behalf of British Society for Neuroendocrinology.

preparations obtained from the rat brain.^{35,37,38} At the cellular level, this isoform has been detected in oligodendrocytes and neurones,³⁹⁻⁴¹ in microglia⁴² and astrocytes,^{39,40} and in Schwann cells.⁴³⁻⁴⁶ Type 2 isoform is widely expressed from the forebrain to the brain stem and cerebellum of the adult rat⁴⁷ and also highly expressed in the spinal cord, particularly in oligodendrocytes.³⁶

Four human 3 α -hydroxysteroid oxidoreductase (HSOR) isozymes, but only one isoform in rats, have been cloned so far.⁴⁸ 3 α -HSOR and 3 β -HSOR has been identified in the central nervous system (CNS)⁴⁹; in particular, 3 α -HSOR has been detected in the rat cerebral cortex, cerebellum⁵⁰ and spinal cord,³⁶ whereas, in the mouse brain, it is co-localised with 5 α -R type 1 in neurones of the cerebral cortex, hippocampus, olfactory bulb, amygdala and thalamus.³⁴ At the cellular level, in addition to neurones, 3 α -HSOR also appears to be highly localised in cultures of type 1 astrocytes^{39,40} and oligodendrocytes.^{36,51} Interestingly, the formation of ALLO by 3 α -HSOR decreases with the differentiation of oligodendrocytes.⁵¹

Interestingly, in the context of the growing literature regarding the role of the gut microbiota-brain axis in human health and disease,⁵²⁻⁵⁷ it is important to highlight that, as recently demonstrated, local steroidogenesis also occurs in the adult male rat colon.⁵⁸ In particular, the levels of ALLO detected in this tissue are significantly higher than those present in plasma. In addition, the mRNA levels of 3 α -HSOR present in the adult male rat colon are significantly higher than those present in the cerebral cortex.⁵⁸

The metabolic conversions by the enzymes 5 α -R, 3 α -HSOR and 3 β -HSOR have a deep impact on the mechanism of action of PROG. Indeed, although DHP, similar to its precursor, is still able to interact with intracellular PROG receptor, ALLO and isallopregnanolone interact with GABA_A receptor. In particular, ALLO is a potent ligand of this non-classical steroid receptor,^{59,60} whereas isallopregnanolone does not bind directly to the GABA_A receptor⁶¹ but, instead, antagonises the effect of ALLO on the GABA_A receptor.^{62,63} In this context, it is important to recall the molecular composition of the GABA_A receptor (Figure 1). This pentameric ionotropic receptor is able to respond differently to benzodiazepines, ALLO or to other modulators depending on the subunit composition. In mammals, it can consist of 19 subunits, grouped in eight classes: α (1-6), β (1-3),

γ (1-3), δ , ϵ , θ , π and ρ (1-3).⁶⁴ In the brain, the most common subunit combination includes two α 1, two β 2 and one γ 2 subunits,^{64,65} with a binding site for modulators placed at the interface between α and β subunits.⁶⁶ Despite the fact that receptors containing the δ subunit, mainly located extrasynaptically, are the most sensitive to neurosteroid modulation,⁶⁷⁻⁶⁹ these molecules, and ALLO in particular, may affect GABA_A receptor function in other ways. For example, they can promote the phosphorylation of α 4 or β 3 subunits.^{70,71} On the other hand, the composition of GABA_A subunits may be altered by continuous administration of PROG or ALLO⁷² (Figure 1). A deeper presentation of GABA_A receptor composition and ligand binding is provided in other recent reviews.⁷²⁻⁷⁴

3 | LEVELS OF ALLO UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

3.1 | Physiological conditions

The first characteristic of ALLO levels is that they may differ in relation to the compartment analysed. This is a consequence of metabolism by 5 α -R and 3 α -HSOR, which is differentially expressed in the nervous system. Thus, ALLO levels show differences between the nervous system, plasma and cerebrospinal fluid (CSF), as well as between the CNS and peripheral nervous system (PNS). Moreover, they also differ between males and females on dioestrus day.⁷⁵ In addition, the levels of PROG metabolites, such as ALLO, its precursor DHP and isallopregnanolone, are higher in the brain of pseudo-pregnant females than in the male brain.⁷⁶

Sex differences in the levels of these PROG metabolites may be the result of a sex dimorphism of the steroidogenic enzymes synthesising these molecules. Indeed, in green anole lizards, 5 α -R type 2 is higher in the brain of females than in the male brain.⁷⁷ In rat cerebellum, 5 α -R is significantly higher in males, whereas 3 α -HSOR is significantly higher in pro-oestrus females than in males.⁵⁰

As observed in gonadectomised animals, the levels of ALLO in the nervous system are also influenced by its circulating levels.

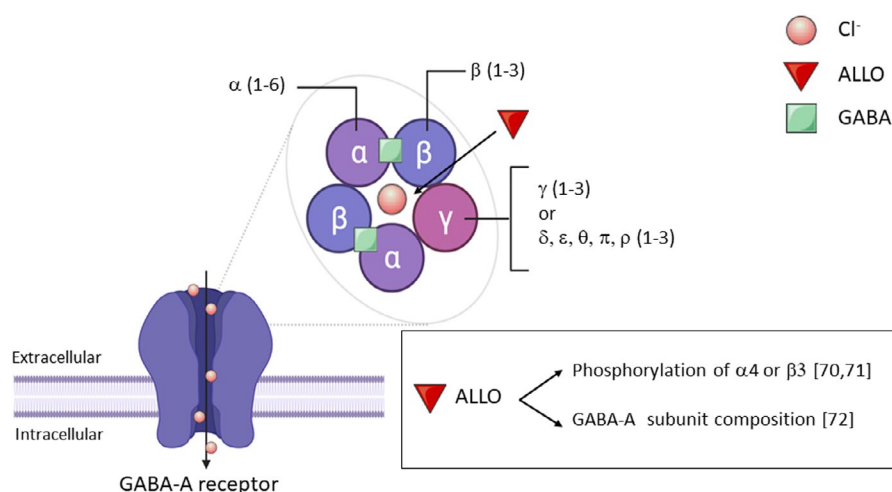


FIGURE 1 GABA_A receptor structure and allopregnanolone mechanism of action. The 19 different subunits of the receptor and the mechanism of action of allopregnanolone are shown. In the box: effects of allopregnanolone on GABA_A receptor subunit composition and phosphorylation are shown. For details, see text. ALLO, allopregnanolone; Cl⁻, chloride

Interestingly, this effect shows specific features in different regions of the nervous system, being different in the two sexes and dependent on the duration of gonadal hormone deprivation.⁷⁸ For example, in both the male cerebral cortex and cerebellum, levels of ALLO are decreased after long-term gonadectomy (ie, 4 months), whereas these effects do not occur in the corresponding structures of the female brain.⁷⁸ Interestingly, as reported recently, 3 α -HSOR expression in the cerebellum is also sex-dimorphic.⁵⁰

This neuroactive steroid is also important during brain development for adolescent and adult behaviour and for nervous system maturation.⁷⁹ Indeed, the levels of ALLO in the forebrain of embryonic rats vary widely throughout development. During the last pregnancy period, ALLO levels sharply increase and decline prior to parturition.⁸⁰ Some of these effects are related to a different functioning of the dorsal hippocampus, probably related to alterations in the expression of GABA receptors containing α 4 and δ subunits, which are molecular alterations that can persist into adult age and can, in part, explain the reported behavioural disturbances.⁸¹

The levels of ALLO in the nervous system, as well as of the other PROG metabolites, are also affected by neurodegenerative and psychiatric disorders. These changes have been demonstrated to be different in males and females, in agreement with many neurodegenerative and psychiatric disorders showing sex-dimorphic features. Some examples of them are discussed in the following subsections.

3.2 | Pathological conditions

3.2.1 | Mood disorders

Several clinical and experimental observations have clearly shown that the plasma and/or CSF levels of ALLO are altered in stress-related disorders and psychiatric diseases, such as anxiety-like behaviour and depression, post-partum depression and post-partum anxiety.⁸²⁻⁹² A decrease in the plasma levels of ALLO has been also observed in association with increased depression and anxiety as well as symptoms in anorexic and overweight/obese women.⁹³ Interestingly, a decrease in the expression of 5 α -R type 1 enzyme has been reported in prefrontal cortex Brodmann's area 9 of depressed patients.⁹⁴

The plasma levels of ALLO are also decreased in human alcoholics,⁹⁵ and are altered after ethanol withdrawal in the mouse cerebral cortex and hippocampus.⁹⁶ In agreement, polymorphic variations in the 3 α -HSOR have been also associated with an increased risk of alcohol dependence.⁹⁷ Interestingly, in this condition, a sex dimorphism of brain ALLO levels has been observed, with higher levels in the substantia nigra pars medialis of men.⁹⁸

Mood disorders, in agreement with their sex dimorphism in term of incidence⁹⁹⁻¹⁰⁴ and/or manifestations,¹⁰⁵⁻¹²⁵ may also alter the levels of ALLO in a sex-dimorphic way. For example, the levels of this neuroactive steroid are decreased in the male, but not the female, brain mouse model of autism spectrum disorder-like behaviour.¹²⁶ In particular, in adult males, a decrease in the levels of this neuroactive

steroid is associated with more severe restricted and repetitive behaviour.¹²⁷

The plasma levels of ALLO are also decreased in association with post-traumatic stress disorders (PTSD) re-experiencing and depressive symptoms in PTSD patients, as well as with enhanced contextual fear memory and impaired fear extinction in PTSD experimental models.^{128,129} Interestingly, in female PTSD patients, the observed low levels of ALLO in the CSF are associated with impairment of the enzyme synthesising this neuroactive steroid (ie, 3 α -HSOR).¹³⁰ However, levels of ALLO are decreased in the medial orbital frontal cortex of male, but not female, PTSD patients.¹³¹

Another interesting example of alteration in ALLO levels is represented by post-finasteride syndrome (PFS). Finasteride (commercially named Propecia or Proscar) is an inhibitor of two isoforms of the 5 α -R (ie, type 1 and 2), although it has higher affinity for type 2 in humans.^{132,133} Approved in 1997 for the treatment of androgenetic alopecia at 1 mg day⁻¹, this drug has been shown to lead to a significant reduction in the progression of baldness and the stimulation of new hair growth.¹³⁴ 5 α -R inhibitors have generally been described as well-tolerated and relatively safe drugs; however, recent observations have led to a more critical re-evaluation of these concepts (Figure 2). Indeed, 5 α -R inhibitors not only induced side effects during the treatment, but also they may persist after drug discontinuation inducing the so named PFS. Among these serious adverse side effects, there are sexual side effects (ie, low libido, erectile dysfunction, decreased arousal and difficulty in achieving orgasm), depression, anxiety and cognitive complaints.¹³⁵ Data obtained in PFS patients show a decrease in the plasma levels of ALLO.¹³⁶ It is interesting to note that, also in an experimental model of PFS, the plasma levels of this neuroactive steroid were decreased. This alteration was associated with a decrease in ALLO levels in the cerebral cortex,¹³⁷ where a decrease in the gene expression of GABA_A receptor α 4 and β 3 subunits was observed¹³⁷ (Figure 2).

3.2.2 | Neurodegenerative disorders

Altered levels of ALLO have been also reported in several neurodegenerative conditions and may differ in the two sexes, according to the sex-dimorphic characteristics of neurodegenerative disorders.^{104,138-144} For example, as reported in the caudate nucleus of Parkinson's disease (PD) patients, the 3 α -HSOR type 3 is up-regulated.¹⁴⁵ In the brain of an experimental model of PD (ie, mouse injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), the levels of ALLO were increased in a manner similar to that occurring in the plasma of these animals.¹⁴⁶ Accordingly, the block in ALLO production by the administration of a 5 α -R inhibitor, such as dutasteride, exerted protective effects on dopamine neurones in the same animal model.¹⁴⁷

Altered levels of ALLO are also detected in patients affected by multiple sclerosis (MS). For example, decreased levels of this neuroactive steroid have been detected in the CSF of relapsing-remitting MS male adult patients,¹⁴⁸ as well as in brain samples of

POST-FINASTERIDE SYNDROME

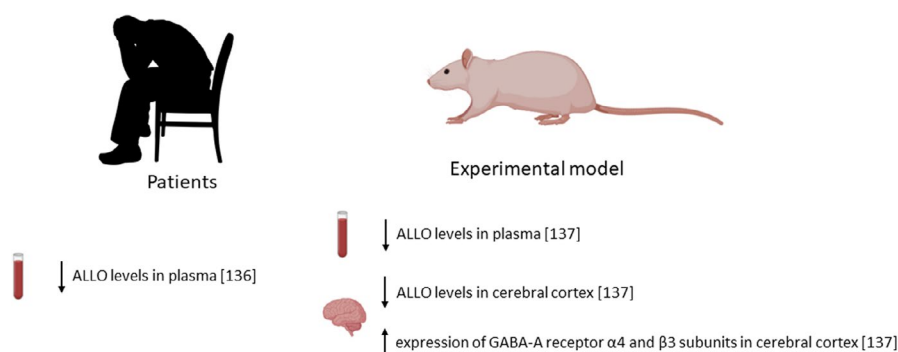


FIGURE 2 Allopregnanolone (ALLO) levels are decreased in plasma of PFS patients, as well as in its experimental model. In the male rat, the levels, as well as the GABA_A receptor composition, are also modified in the cerebral cortex. For details, see text. ALLO, allopregnanolone; GABA, γ -aminobutyric acid; PFS, post-finasteride syndrome

male MS patients.¹⁴⁹ Observations in an experimental model of MS, such as the experimental autoimmune encephalomyelitis (EAE) rat MS model,^{150,151} confirmed alterations in ALLO levels. Interestingly, these changes depend on the pathological phase considered, as well as the sex. For example, the levels of this neuroactive steroid increase at the acute phase of the disease (ie, 14 days post-immunisation) in the spinal cord of males, but not females.¹⁵⁰ By contrast, at the chronic phase (ie, 40 days post-immunisation), no changes were reported in both sexes.¹⁵¹ The pattern in plasma is different. Indeed, at the acute phase, ALLO levels are decreased in females, but not males,¹⁵⁰ whereas, at the chronic phase, the ALLO plasma levels are increased only in male animals.¹⁵¹ The levels of ALLO were altered in a sex-dimorphic way also depending on the nervous region considered. Indeed, in the female, but not the male, cerebellum, ALLO levels are decreased both at the acute¹⁵⁰ and chronic phase of the disease.¹⁵¹ In the male, but not the female, cerebral cortex, an increase in the levels of ALLO was observed at the acute phase of the disease.¹⁵⁰ At the chronic phase, the levels of this neuroactive steroid were unaffected in the cerebral cortex of male and female rats.¹⁵¹ Sex differences in ALLO levels have been also detected in human relapsing-remitting MS patients. Indeed, ALLO levels in the CSF are higher in male than in female patients.¹⁵² However, this difference is observed in the active, but not the stable, phase, where the levels are comparable in the two sexes.¹⁵²

Brain levels of ALLO have been reported to be affected in a sex-dimorphic way also in an experimental model of traumatic brain injury (TBI).^{76,153,154} Indeed, TBI decreased the brain levels of this neuroactive steroid in female mice,¹⁵³ but not male mice.¹⁵⁴

Diabetes mellitus alters central (ie, diabetic encephalopathy), as well as peripheral (ie, diabetic peripheral neuropathy), nervous function. ALLO levels are decreased in the cerebral cortex of both long-term (ie, 3 months post-induction) diabetic male and female rats.¹⁵⁵ By contrast, the levels of this neuroactive steroid are decreased in the spinal cord of diabetic males, but not diabetic females.¹⁵⁵ Long-term diabetes also induced a decrease in ALLO levels in a peripheral nerve, such as the sciatic nerve, with altered levels in female animals, but not male animals.¹⁵⁵ Similar to that reported in MS, and also in case of diabetes mellitus, alterations in the ALLO levels depend on the pathological phase considered. Indeed, short-term diabetes (ie, 1 month postinduction) induces a decrease in the levels of this

neuroactive steroid in the cerebral cortex and hippocampus of male animals.^{156,157} In addition, an increase in the ALLO levels occurs only in the diabetic male sciatic nerve.¹⁵⁸

Altered levels of this neuroactive steroid have been also reported in other animal models of peripheral neuropathy. For example, in the sciatic nerve of the sterol regulatory element binding protein-1C knockout mice, a model of peripheral neuropathy as a result of the ablation of the key lipogenic transcription factor,¹⁵⁹ the levels of ALLO are increased at 10 months of age compared to those observed in wild-type animals.¹⁶⁰ The crush injury of the rat sciatic nerve induced a decrease in the levels of ALLO, in agreement with the reduced levels of its precursor, DHP. These events may be associated with a decrease in the expression of enzyme 5 α -R in the distal portion of the injured nerve.¹⁶¹

An important component of the peripheral neuropathy is the neuropathic pain. As demonstrated in an animal model of neuropathic pain induced by peripheral nerve injury, the levels of ALLO are increased in the spinal cord, together with increased expression and activity of 3 α -HSOR.¹⁶² As proposed, the increase in the levels of neuroactive steroid and its synthesising enzyme, 3 α -HSOR, appears to be an adaptive response to cope with pain.^{163,164} Indeed, an increase in ALLO levels has been reported in the rat lateral thalamus (ie, an important brain region for pain modulation) after spared nerve injury.¹⁶⁵

4 | EFFECTS OF ALLO UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

4.1 | Physiological effects

ALLO regulates lordosis and other motivated behaviours¹⁶⁶ by its action on GABA_A receptors located in the midbrain ventral tegmental area.^{167,168} However, this action appears to be mediated not only by this neurotransmitter receptor, but also by PROG receptor because the administration of mifepristone (ie, an antagonist of PROG receptor) inhibits the induction of this behavioural response to ALLO.^{169,170} This effect can be explained based on the ability of ALLO to be retro-converted into DHP by 3 α -HSOR.¹⁷¹⁻¹⁷³

A critical role for ALLO has been also demonstrated in brain maturation. The physiological fluctuations of this steroid occurring during rodent fetal life and after birth⁸⁰ may contribute to maintaining the low level of arousal activity, characteristic of fetal brain.¹⁷⁴ In addition, neonatal levels of ALLO promote the formation of neuronal circuitry and support the survival of developing neurones.¹⁷⁵ Moreover, this neuroactive steroid is involved in the structural formation of the cerebral cortex, thalamus and hippocampus.^{176,177} Furthermore, ALLO is involved in myelin formation of the CNS.¹² However, this neuroactive steroid is not only important for brain fetal maturation, but also for the pregnant mother. Indeed, during pregnancy, an increase of ALLO levels occurs in the maternal peripheral circulation, as well as in the maternal brain.^{178,179} In rats, the increased levels of this neuroactive steroid interfere with the hypothalamic-pituitary-adrenal (HPA) axis reducing, in particular during late pregnancy, the response to stress exposure of the mother.^{180–183}

ALLO exerts a crucial role also in the adult brain. At this stage, the enzymatic complex 5 α -R/3 α -HSOR co-localises in glutamatergic and GABAergic neurones of the cerebral cortex, hippocampus, amygdala and thalamus, suggesting that its activity is relevant for the synthesis and the effects of neurotransmitters in these cells.³⁴ Indeed, ALLO is able to increase the protein content of glutamic acid decarboxylase in the olfactory bulb.¹⁸⁴ In addition, this steroid regulates the neuronal cytoskeleton because its administration to ovariectomised animals decreases microtubule-associated protein Tau and glycogen synthase kinase 3 β expression in the cerebellum.¹⁸⁵

ALLO is also involved in the mood regulation. For example, together with glucocorticoids, this neuroactive steroid regulates the stress response. Thus, an increase in the ALLO levels has been reported in plasma and cerebral cortex of adult male rats after swim stress.¹⁸⁶

ALLO is also able to regulate the dopaminergic system.^{187–190} In an experimental model in which dopaminergic signalling was altered (ie, animals reared in social isolation), a decrease in the levels of ALLO occurred in the brain but not in plasma.¹⁹¹ In addition, in the foot shock stress model, treatment with this neuroactive steroid stimulates the extracellular dopamine release from cortical dopaminergic neurones,¹⁹² and prevents the dopamine increase in the cerebral cortex and in the nucleus accumbens.¹⁹³ Moreover, ALLO modulates the levels and metabolism of this neurotransmitter during the oestrous phase of the female ovarian cycle. Indeed, it decreases the levels of dopamine and the dopamine metabolite 3,4-dihydroxyphenylacetic acid in the striatum,¹⁹⁴ as well as the dopamine output in the nucleus accumbens and prefrontal cortex in freely moving rats.¹⁹³ In addition, females showing high progesterone levels (ie, in pro-oestrus) are less responsive to ALLO treatment than in other oestrous phases.¹⁹⁵

Interestingly, it has been proposed that ALLO may also affect the enzymatic activity of the DNA base excision repair (BER) pathway. Indeed, as recently reported in both natural and stressful conditions, the treatment with this neuroactive steroid is able to modulate the synthesis of BER pathway enzymes in sheep hippocampus and amygdala.¹⁹⁶

Physiological effects of ALLO have been also reported in the PNS. In Schwann cells, ALLO treatment enhances GABA synthesis through an increased expression of glutamic acid decarboxylase¹⁹⁷ and also promotes glutamate uptake through an increase in the excitatory amino acid carrier 1.¹⁹⁸ ALLO treatment is also able to regulate, in peripheral nerves and Schwann cells, the expression of specific transcription factors involved in the myelination process (ie, Krox-20)¹⁹⁹ and the expression of a myelin protein (ie, peripheral myelin protein 22, PMP22).^{200,201} An antagonist of the GABA_A receptor, such as the bicuculline, is able to completely abolish the stimulatory effect exerted by ALLO on PMP22 in Schwann cell cultures. In addition, a GABA_A receptor agonist (ie, muscimol) shows a stimulatory effect on PMP22 that was comparable to that of ALLO.²⁰² These observations, together with the finding that peripheral nerves, as well as Schwann cells, express GABA_A receptors,^{15,200} may suggest that the effect on peripheral myelin are mediated by the GABA_A receptor.^{200,201} Indeed, isoallopregnanolone, which does not directly interact with GABA_A receptor, does not alter PMP22 expression. Interestingly, the effect of ALLO on the expression of myelin proteins is sex-dimorphic. Indeed, the treatment with this neuroactive steroid increases the expression of PMP22 and of another myelin protein, such as glycoprotein zero, in female rat Schwann cells, but not in male cells.²⁰³

4.2 | Effects of ALLO in pathological conditions

The therapeutic potential of ALLO has been explored in different pathological conditions, demonstrating interesting beneficial effects (Figure 3). ALLO treatment exerts anxiolytic and anti-stress actions.^{204,205} Activation of GABA_A receptors by this neuroactive steroid appears to be responsible for these effects.²⁰⁶ Interestingly, corticotrophin-releasing hormone (CRH) neurones, the primary regulators of the HPA axis, are regulated by GABAergic inhibition.²⁰⁷ In particular, it has been shown that CRH neurones are controlled by delta (δ)-containing GABA_A receptors.²⁰⁸ In agreement, *in vitro* studies showed that the human CRH promoter activity was inhibited by ALLO after basal or forskolin-induced promoter activity.²⁰⁹ In addition, in virgin female rats, ALLO administration was able to reduce CRH gene expression in the parvocellular paraventricular nucleus.¹⁸⁰ Similarly, recent evidence in sheep has demonstrated that, in stressful conditions, this neuroactive steroid reduced CRH gene expression, as well as pro-opiomelanocortin expression, in anterior pituitary, resulting in diminished levels of plasma adrenocorticotrophic hormone and cortisol.²¹⁰ By contrast, the anti-depressive effect exerted by ALLO, at least in the forced swimming model, appears also to involve the stimulation of dopamine D2-like receptors.²¹¹ In addition, it has been observed that, in the nucleus accumbens of learned helplessness rats (ie, an experimental model of depression), the astroglial glutamate transporter-1 and glutamine synthetase system is normalised by ALLO treatment.²¹² In this context, it is interesting to note that effective antidepressant treatment is able to increase the reduced levels of ALLO observed in depressed

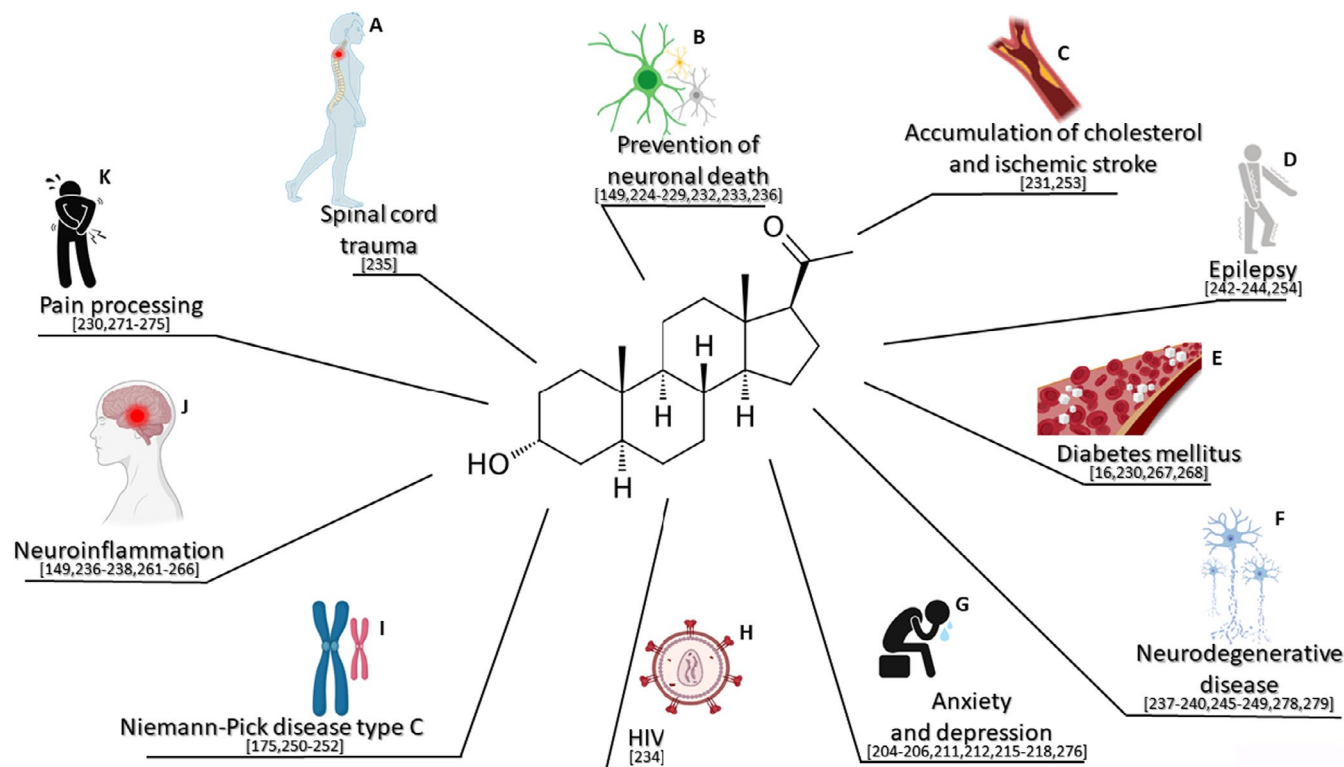


FIGURE 3 Neuroprotective effects of allopregnanolone. Treatment with this neuroactive steroid shows: (A) beneficial effects on spinal cord trauma, (B) prevention of neuronal death, (C) reduction of cholesterol accumulation and stroke, (D) decrease in epileptic events, (E) beneficial effects in nervous damage induced by diabetes mellitus, (F) protective effects on neurodegenerative diseases (eg, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis), (G) anxiolytic and anti-stress actions, (H) effects against the neurotoxicity exerted by human immunodeficiency virus (HIV), (I) protective effects in an experimental model of Niemann-Pick type C and in (J) neuroinflammatory conditions (eg, multiple sclerosis and experimental autoimmune encephalomyelitis), and (K) analgesic effects against neuropathic pain

patients.²¹³ In agreement, in an experimental model, the antidepressant fluoxetine was able to increase ALLO levels.²¹⁴ Interestingly, in mood and anxiety disorders, ALLO treatment shows sex-specific features. Indeed, this neuroactive steroid attenuates in females, but not in males, the HPA axis responses to interleukin-1 β in adult prenatally stressed rats.²¹⁵ Also only in females, ALLO treatment blocks the stress-induced reinstatement of cocaine-seeking behaviour induced by yohimbine.²¹⁶ ALLO treatment before stress reduced basal CRF mRNA expression in male rats.²¹⁷ Interestingly, recent observations obtained in rats show sex- and brain region-specific regulation of CRF after ALLO treatment, suggesting new sex-specific therapeutic approaches based on this neuroactive steroid for stress-related disorders and addiction.²¹⁸

Despite ALLO treatment shows anxiolytic effects, women with premenstrual dysphoric disorder show an altered sensitivity to this neuroactive steroid over the menstrual cycle compared to healthy controls.²¹⁹ In these patients, the negative mood symptoms are antagonised by isoallopregnanolone treatment in the premenstrual phase, reducing negative mood symptoms in premenstrual dysphoric disorder.²²⁰ As suggested, a possible hypothesis for this paradoxical effect could be changes in GABA_A receptor composition (ie, an up-regulation of the $\alpha 4$, β , δ subunit expression) during the luteal phase.²²¹

Similarly, in D1CT-7 mice (ie, an experimental model of Tourette syndrome), ALLO treatment exacerbated the Tourette syndrome symptoms,²²² whereas isoallopregnanolone administration is able to reduce the number of tic-like behaviours induced by stress.²²³

ALLO treatment has also been reported to exert protective effects in experimental models of neurodegeneration. For example, this neuroactive steroid is protective against kainic acid-induced excitotoxicity in the hippocampus in vivo,²²⁴ reduces seizures,²²⁵⁻²²⁹ prevents cell apoptosis in the spinal cord of streptozotocin (STZ) diabetic rats,²³⁰ and protects against stroke,²³¹ oxygen-glucose deprivation,²³² TBI²³³ and the neurotoxic effects exerted by human immunodeficiency virus.²³⁴

ALLO treatment exerts protective effect also in spinal cord trauma. For example, in organotypic spinal cord cultures put under injury (ie, a weight drop model), this neuroactive steroid, by activation of GABA_A receptors, is able to decrease membrane damage and prevent neuronal death.²³⁵

ALLO is also effective in experimental model of MS, such as EAE, where the treatment reduces axonal injury,^{149,236} as well as in Alzheimer's disease (AD) models, where it is able to induce neurogenesis/oligodendrogenesis and to reduce β -amyloid levels^{237,238} and bioenergetics deficits.²³⁹ In particular, for the neuroprotective effects of i.v. ALLO treatment in AD, the dosing and treatment

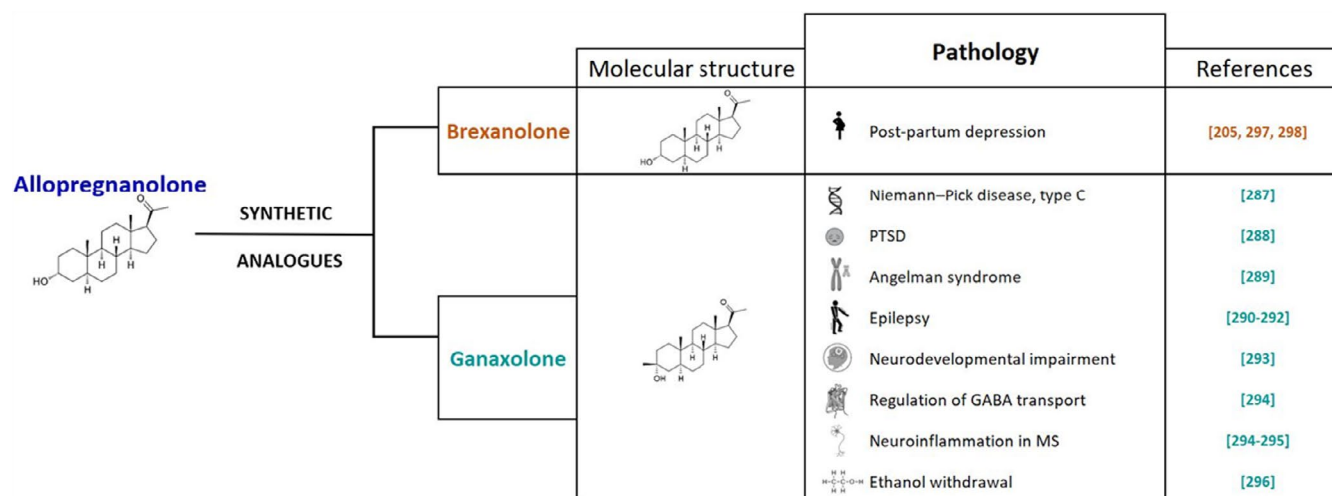


FIGURE 4 Protective effects exerted by allopregnanolone analogues, brexanolone and ganaxolone, in different neuropathologies. For details see text. PTSD, post-traumatic stress disorder; MS, multiple sclerosis

regimen appears to be crucial.^{237,238,240} By contrast, intranasal delivery of this neuroactive steroid has been proposed as an excellent therapeutic strategy against seizures.²⁴¹ In this context, it is important to highlight that neuroactive steroids represent an important target for the treatment of focal epileptic disorders.²⁴² Indeed, alteration of ALLO synthesis modulate status epilepticus dynamics.^{243,244} In addition, protective effects have been reported in an experimental model of amyotrophic lateral sclerosis (ie, Wobbler mouse),²⁴⁵ in PD experimental models,^{246,247} as well as in a pilot clinical study performed in patients affected by fragile X-associated tremor/ataxia syndrome, where the ALLO treatment was reported to improve cognitive function and neurodegeneration.^{248,249}

In an animal model of Niemann-Pick type C disease, this neuroactive steroid has been demonstrated to delay the onset of neurological symptoms, increasing Purkinje and granule cell survival in the cerebellum, reducing cortical ganglioside accumulation, cholesterol accumulation and inflammation, and enhancing myelination.^{175,250,251} Interestingly, the combination of this neuroactive steroid with cyclodextrin and miglustat seems to ameliorate motor but not cognitive deficits.²⁵²

Few experimental observations have been performed to evaluate possible sex difference in the protective effects of ALLO on neurodegeneration. As demonstrated, a low dose of this neuroactive steroid induces a higher neuroprotection from ischaemic damage in females compared to males.²⁵³ In an animal model of epilepsy, the treatment shows greater antiseizure potency in females than in males; this effect was associated with higher levels of extrasynaptic delta subunit of GABA_A receptors in female animals.²⁵⁴

An important aspect in neurodegenerative and psychiatric diseases is the neuroinflammation.²⁵⁵⁻²⁶⁰ Indeed, ALLO exerts a variety of protective effects in this process. For example, this neuroactive steroid reduces protein-protein interactions initiating toll-like receptor 4 (TLR4)-dependent signalling in immune cells and the brain²⁶¹ alongside of TLR7.²⁶² In addition, its treatment decreases microglia reactivity and lymphocyte infiltration in an EAE

experimental model,^{149,236} as well as neuroinflammatory burden in AD models.^{237,238} A protective effect has been also reported in ischaemic stroke, where its treatment down-regulates the production of pro-inflammatory cytokines (ie, tumour necrosis factor- α and interleukin-6) protecting against blood-brain barrier disruption and reducing infarct size.²⁶³ Finally, after TBI, ALLO decreases the expression levels of interleukin-1 β and tumour necrosis factor- α , in the rat brain,²⁶⁴ and increases a potent inhibitor of the complement convertases that are activators of the inflammatory cascade.²⁶⁵ Indeed, it has been recently demonstrated that administration of this neuroactive steroid to primary cell cultures or to a microglial cell line (ie, BV-2), induces changes in morphology and phagocytic activity in microglial cells.²⁶⁶ These results might help to shed light on the protective mechanisms of ALLO in inflammatory conditions. Protective effects of ALLO have been also reported in peripheral neuropathies. For example, in an experimental model of peripheral diabetic neuropathy (ie, rats rendered diabetic by streptozotocin injection), ALLO treatment improves nerve conduction velocity, thermal threshold, mRNA levels of a myelin protein such as PMP22, and skin innervation density.²⁶⁷ In addition, this neuroactive steroid is also able to counteract myelin abnormalities in rat peripheral nerves induced by the ageing process.^{16,268}

Neuropathic pain is another important component of the damage in the PNS and CNS. In this context, it is important to note that 3 α -HSOR is expressed in pain information processing areas of the CNS, such as the dorsal root ganglia and the dorsal horn of the spinal cord.^{163,269} Indeed, blockade of 3 α -HSOR and the consequent inhibition of the local synthesis of THP in these two compartments enhances neuropathic pain induced by sciatic nerve injury.^{163,164} In addition, the synthesis of ALLO in the dorsal horn of the spinal cord is regulated by an important neuropeptide involved in pain processing, such as substance P.²⁷⁰ Altogether, these observations suggest that endogenous ALLO is involved in pain processing. From this point of view, ALLO exerts analgesic effects. For example, treatment with this neuroactive steroid ameliorates diabetic-induced thermal

hyperalgesia in the STZ model.²³⁰ In addition, it suppresses allodynia/hyperalgesia evoked by antineoplastic drugs, such as vincristine²⁷¹ or oxaliplatin,²⁷² or by spinal nerve ligation.²⁷³ The analgesic actions of ALLO appear to be mediated by the potentiation of GABA_A receptor activity and the inhibition of T-type Ca²⁺ channels.^{274,275}

Altogether, these observations indicate that ALLO may be considered as a potential candidate for the treatment of psychiatric,²⁷⁶ traumatic²⁷⁷ and neurodegenerative disorders.^{278,279} However, one of the disadvantages of the treatment with natural ALLO is represented by its rapid metabolism and their low oral bioavailability.⁸⁷ On this basis, extensive research has been devoted to synthesising analogues of ALLO,²⁸⁰⁻²⁸³ showing promising neuroprotective effects.^{205,277,284-286} In particular, as depicted in Figure 4, two synthetic analogues, such as ganaxolone and brexanolone, appear to be very promising. Indeed, ganaxolone has been demonstrated to be neuroprotective in an experimental model of Niemann-Pick type C,²⁸⁷ in an animal model of PTSD,²⁸⁸ in Angelman syndrome,²⁸⁹ and in animal models of epilepsy and related conditions.²⁹⁰⁻²⁹² In addition, its treatment is able to reduce neurodevelopmental impairment following preterm birth,²⁹³ to regulate GABA transport and neuroinflammation in MS,²⁹⁴ to induce remyelination in focal demyelination of the corpus callosum²⁹⁵ and to be effective for the treatment of ethanol withdrawal-induced seizures.²⁹⁶

Brexanolone has been recently approved by the US Food and Drug Administration for the specific treatment of post-partum depression,^{205,297,298} even some concerns regarding its use have been also raised^{299,300} (Figure 4).

An alternative to the use of synthetic steroids is to stimulate the endogenous synthesis of ALLO. One option is the activation of steroidogenesis with ligands of TSPO, a part of the macromolecular complex involved in the transfer of cholesterol into mitochondria (ie, the first step of the steroidogenesis).³⁰¹ Indeed, treatment with TSPO ligands has been reported to exert neuroprotective effects, such as in EAE mice using etifoxine³⁰² or XBD173,³⁰³ in rat models of PTSD administered with midazolam³⁰⁴ or YL-IPA08,³⁰⁵ in a rat *ex vivo* glaucoma model with PK11195³⁰⁶ and in diabetic rats with Ro5-4864^(307,308) or AC-5216.³⁰⁹

Another possibility is the activation of liver X receptors (LXRs). Indeed, treatment with a LXR ligand such as the GW3965 increases the levels of ALLO in the spinal cord and the cerebral cortex, as well as the levels of its precursor, DHP, in the sciatic nerve of diabetic rats.^{307,310} This, in turn, exerts neuroprotective effects on thermal nociceptive activity, nerve conduction velocity and Na⁺,K⁺-ATPase activity.³¹⁰

5 | CONCLUSIONS

As defined many decades ago,³¹¹ neuroactive steroids represent important physiological modulators of the nervous system. They are involved in basic processes such as myelination, neuronal transmission and brain maturation. Among the natural neuroactive steroids, ALLO has received particular attention because of its relevance in

such processes. Concerning ALLO physiology, many issues have to be taken into account. For example, its levels are linked to the expression of the enzymatic complex of 5 α -R/3 α -HSOR, thus producing a different profile in relation to the nervous structure being considered. In addition, neuroactive steroid plasma levels, as well as the sex, have an influence on the levels of ALLO in the nervous system.

In addition, as more recently explored, the neuroactive steroids are also neuroprotective agents. Among them, ALLO appears to be particularly relevant because of its implication in neuropathological situations. Up to now, its importance in depression and anxiety, in neurodegenerative diseases (eg, AD, PD and diabetes mellitus), in traumatic events (eg, spinal cord trauma, nerve injury), and in inflammatory environments (eg, MS, ischaemia), is becoming increasingly evident. ALLO exerts its protective effects mainly by interaction with the GABA_A receptor, although, as a result of the ability of the enzyme 3 α -HSOR to retro-convert ALLO into DHP, this steroid may also interact with PROG receptor. The unfavourable pharmacokinetic of ALLO limits its therapeutic potential, as observed in many experimental paradigms. Thus, alternative strategies have been explored. For example, synthetic analogues have been successfully applied to several pathological conditions, also leading to its inclusion in clinical practice. An alternative to the synthetic ALLO derivative administration is represented by the pharmacological stimulation of steroidogenesis, and consequently ALLO synthesis, by specific ligands.

In conclusion, a deeper investigation of the mechanisms involved in the protective effects of neuroactive steroids in general, and of ALLO in particular, is needed to propose new therapeutic strategies based on this neuroactive steroid for the treatment of neuropathological conditions.

ACKNOWLEDGEMENTS

This research was supported by grants from MIUR Progetto Eccellenza and PON "Ricerca e Innovazione" PerMedNet -project ARS01_01226; The financial support of PRIN (2017ZFJCS3) and Intramural Grant Line-2 Action-A (PSR2020) from Università degli Studi di Milano to Silvia Giatti is also gratefully acknowledged.

AUTHOR CONTRIBUTIONS

Silvia Diviccaro: Conceptualisation; Writing – original draft; Writing – review & editing. **Lucia Cioffi:** Writing – review & editing. **Eva Falvo:** Visualisation. **Silvia Giatti:** Conceptualisation; Writing-original draft; Writing – review & editing. **Roberto Cosimo Melcangi:** Conceptualisation; Writing-original draft; Writing – review & editing.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jne.12996>.

ORCID

Roberto Cosimo Melcangi  <https://orcid.org/0000-0003-0861-8967>

REFERENCES

- Micevych P, Sinchak K. The neurosteroid progesterone underlies estrogen positive feedback of the LH surge. *Front Endocrinol (Lausanne)*. 2011;2:90.
- Micevych P, Sinchak K. Synthesis and function of hypothalamic neuroprogesterone in reproduction. *Endocrinology*. 2008;149(6):2739-2742.
- Skinner DC, Evans NP, Delaleu B, Goodman RL, Bouchard P, Caraty A. The negative feedback actions of progesterone on gonadotropin-releasing hormone secretion are transduced by the classical progesterone receptor. *Proc Natl Acad Sci USA*. 1998;95(18):10978-10983.
- Barracough CA, Camp P, Weiland N, Akabori A. Stimulatory versus inhibitory effects of progesterone on estrogen-induced phasic LH and prolactin secretion correlated with estrogen nuclear and progesterone cytosol receptor concentrations in brain and pituitary gland. *Neuroendocrinology*. 1986;42(1):6-14.
- Banks JA, Freeman ME. Inhibition of the daily LH release mechanism by progesterone acting at the hypothalamus. *Biol Reprod*. 1980;22(2):217-222.
- Wang JM, Liu L, Irwin RW, Chen S, Brinton RD. Regenerative potential of allopregnanolone. *Brain Res Rev*. 2008;57(2):398-409.
- Giachino C, Galbiati M, Fasolo A, Peretto P, Melcangi R. Neurogenesis in the subependymal layer of the adult rat: a role for neuroactive derivatives of progesterone. *Ann NY Acad Sci*. 2003;1007:335-339.
- Tsutsui K. Neurosteroid biosynthesis and action during cerebellar development. *Cerebellum*. 2012;11(2):414-415.
- Tsutsui K, Ukena K, Sakamoto H, Okuyama S, Haraguchi S. Biosynthesis, mode of action, and functional significance of neurosteroids in the purkinje cell. *Front Endocrinol (Lausanne)*. 2011;2:61.
- Luquin S, Naftolin F, Garcia-Segura LM. Natural fluctuation and gonadal hormone regulation of astrocyte immunoreactivity in dentate gyrus. *J Neurobiol*. 1993;24(7):913-924.
- Garcia-Segura LM, Luquin S, Parducz A, Naftolin F. Gonadal hormone regulation of glial fibrillary acidic protein immunoreactivity and glial ultrastructure in the rat neuroendocrine hypothalamus. *Glia*. 1994;10(1):59-69.
- Ghoumari AM, Ibanez C, El-Etr M, et al. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. *J Neurochem*. 2003;86(4):848-859.
- Ghoumari AM, Baulieu EE, Schumacher M. Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. *Neuroscience*. 2005;135(1):47-58.
- Schumacher M, Hussain R, Gago N, Oudinet JP, Mattern C, Ghoumari AM. Progesterone synthesis in the nervous system: implications for myelination and myelin repair. *Front Neurosci*. 2012;6:10.
- Melcangi RC, Giatti S, Pesaresi M, et al. Role of neuroactive steroids in the peripheral nervous system. *Front Endocrinol (Lausanne)*. 2011;2:104.
- Melcangi RC, Azcoitia I, Ballabio M, et al. Neuroactive steroids influence peripheral myelination: a promising opportunity for preventing or treating age-dependent dysfunctions of peripheral nerves. *Prog Neurobiol*. 2003;71(1):57-66.
- Chan JR, Rodriguez-Waitkus PM, Ng BK, Liang P, Glaser M. Progesterone synthesized by Schwann cells during myelin formation regulates neuronal gene expression. *Mol Biol Cell*. 2000;11(7):2283-2295.
- Chan JR, Phillips LJ 2nd, Glaser M. Glucocorticoids and progestins signal the initiation and enhance the rate of myelin formation. *Proc Natl Acad Sci USA*. 1998;95(18):10459-10464.
- Stein DG. Progesterone in the treatment of acute traumatic brain injury: a clinical perspective and update. *Neuroscience*. 2011;191:101-106.
- Ibanez C, Shields SA, El-Etr M, et al. Steroids and the reversal of age-associated changes in myelination and remyelination. *Prog Neurobiol*. 2003;71(1):49-56.
- Melcangi RC, Garcia-Segura LM. Sex-specific therapeutic strategies based on neuroactive steroids: in search for innovative tools for neuroprotection. *Horm Behav*. 2010;57:2-11.
- Giatti S, Caruso D, Boraso M, et al. Neuroprotective effects of progesterone in chronic experimental autoimmune encephalomyelitis. *J Neuroendocrinol*. 2012;24:851-861.
- Garay L, Deniselle MC, Meyer M, et al. Protective effects of progesterone administration on axonal pathology in mice with experimental autoimmune encephalomyelitis. *Brain Res*. 2009;1283:177-185.
- Garay L, Deniselle MC, Lima A, Roig P, De Nicola AF. Effects of progesterone in the spinal cord of a mouse model of multiple sclerosis. *J Steroid Biochem Mol Biol*. 2007;107(3-5):228-237.
- Callier S, Morissette M, Grandbois M, Pelaprat D, Di Paolo T. Neuroprotective properties of 17beta-estradiol, progesterone, and raloxifene in MPTP C57Bl/6 mice. *Synapse*. 2001;41(2):131-138.
- Bourque M, Dluzen DE, Di Paolo T. Neuroprotective actions of sex steroids in Parkinson's disease. *Front Neuroendocrinol*. 2009;30(2):142-157.
- Brinton RD. Neurosteroids as regenerative agents in the brain: therapeutic implications. *Nat Rev Endocrinol*. 2013;9(4):241-250.
- Melcangi RC, Garcia-Segura LM, Mensah-Nyagan AG. Neuroactive steroids: state of the art and new perspectives. *Cell Mol Life Sci*. 2008;65(5):777-797.
- Pelletier G. Steroidogenic enzymes in the brain: morphological aspects. *Prog Brain Res*. 2010;181:193-207.
- Stiles AR, Russell DW. SRD5A3: a surprising role in glycosylation. *Cell*. 2010;142(2):196-198.
- Normington K, Russell DW. Tissue distribution and kinetic characteristics of rat steroid 5 alpha-reductase isozymes. Evidence for distinct physiological functions. *J Biol Chem*. 1992;267(27):19548-19554.
- Russell DW, Wilson JD. Steroid 5 alpha-reductase: two genes/two enzymes. *Annu Rev Biochem*. 1994;63:25-61.
- Celotti F, Melcangi RC, Martini L. The 5 alpha-reductase in the brain: molecular aspects and relation to brain function. *Front Neuroendocrinol*. 1992;13(2):163-215.
- Agis-Balboa RC, Pinna G, Zhubi A, et al. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci USA*. 2006;103(39):14602-14607.
- Melcangi RC, Celotti F, Ballabio M, et al. Ontogenetic development of the 5 alpha-reductase in the rat brain: cerebral cortex, hypothalamus, purified myelin and isolated oligodendrocytes. *Brain Res Dev Brain Res*. 1988;44(2):181-188.
- Patte-Mensah C, Penning TM, Mensah-Nyagan AG. Anatomical and cellular localization of neuroactive 5 alpha/3 alpha-reduced steroid-synthesizing enzymes in the spinal cord. *J Comp Neurol*. 2004;477(3):286-299.
- Melcangi RC, Celotti F, Ballabio M, Poletti A, Castano P, Martini L. Testosterone 5 alpha-reductase activity in the rat brain is highly concentrated in white matter structures and in purified myelin sheaths of axons. *Journal of steroid biochemistry*. 1988;31(2):173-179.
- Melcangi RC, Celotti F, Ballabio M, Carnaghi R, Poletti A, Martini L. Effect of postnatal starvation on the 5 alpha-reductase activity of the brain and of the isolated myelin membranes. *Exp Clin Endocrinol*. 1989;94(3):253-261.
- Melcangi RC, Celotti F, Castano P, Martini L. Differential localization of the 5 alpha-reductase and the 3 alpha-hydroxysteroid dehydrogenase in neuronal and glial cultures. *Endocrinology*. 1993;132(3):1252-1259.
- Melcangi RC, Celotti F, Martini L. Progesterone 5-alpha-reduction in neuronal and in different types of glial cell cultures: type 1 and 2 astrocytes and oligodendrocytes. *Brain Res*. 1994;639(2):202-206.

41. Melcangi RC, Celotti F, Ballabio M, et al. 5 alpha-reductase activity in isolated and cultured neuronal and glial cells of the rat. *Brain Res.* 1990;516(2):229-236.
42. Gottfried-Blackmore A, Sierra A, Jellinck PH, McEwen BS, Bulloch K. Brain microglia express steroid-converting enzymes in the mouse. *J Steroid Biochem Mol Biol.* 2008;109(1-2):96-107.
43. Melcangi RC, Celotti F, Ballabio M, Poletti A, Martini L. Testosterone metabolism in peripheral nerves: presence of the 5 alpha-reductase-3 alpha-hydroxysteroid-dehydrogenase enzymatic system in the sciatic nerve of adult and aged rats. *Journal of steroid biochemistry.* 1990;35(1):145-148.
44. Yokoi H, Tsuruo Y, Ishimura K. Steroid 5alpha-reductase type 1 immunolocalized in the rat peripheral nervous system and paraventricular ganglia. *Histochem J.* 1998;30(10):731-739.
45. Melcangi RC, Magnaghi V, Galbiati M, Martini L. Formation and effects of neuroactive steroids in the central and peripheral nervous system. *Int Rev Neurobiol.* 2001;46:145-176.
46. Schaeffer V, Meyer L, Patte-Mensah C, Mensah-Nyagan AG. Progress in dorsal root ganglion neurosteroidogenic activity: basic evidence and pathophysiological correlation. *Prog Neurobiol.* 2010;92(1):33-41.
47. Castelli MP, Casti A, Casu A, et al. Regional distribution of 5alpha-reductase type 2 in the adult rat brain: an immunohistochemical analysis. *Psychoneuroendocrinology.* 2013;38(2):281-293.
48. Penning TM, Jin Y, Heredia VV, Lewis M. Structure-function relationships in 3alpha-hydroxysteroid dehydrogenases: a comparison of the rat and human isoforms. *J Steroid Biochem Mol Biol.* 2003;85(2-5):247-255.
49. Melcangi RC, Giatti S, Calabrese D, et al. Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. *Prog Neurobiol.* 2014;113:56-69.
50. Giatti S, Diviccaro S, Garcia-Segura LM, Melcangi RC. Sex differences in the brain expression of steroidogenic molecules under basal conditions and after gonadectomy. *J Neuroendocrinol.* 2019;31:e12736.
51. Gago N, Akwa Y, Sananes N, et al. Progesterone and the oligodendroglial lineage: stage-dependent biosynthesis and metabolism. *Glia.* 2001;36(3):295-308.
52. Sharon G, Sampson TR, Geschwind DH, Mazmanian SK. The Central Nervous system and the gut microbiome. *Cell.* 2016;167(4):915-932.
53. Lerner A, Neidhofer S, Matthias T. The gut microbiome feelings of the brain: a perspective for non-microbiologists. *Microorganisms.* 2017;5(4):66.
54. Mayer EA. Gut feelings: the emerging biology of gut-brain communication. *Nat Rev Neurosci.* 2011;12(8):453-466.
55. Lubomski M, Tan AH, Lim SY, Holmes AJ, Davis RL, Sue CM. Parkinson's disease and the gastrointestinal microbiome. *J Neurol.* 2019.
56. Fung C, Vanden BP. Functional circuits and signal processing in the enteric nervous system. *Cell Mol Life Sci.* 2020;77(22):4505-4522.
57. Yissachar N. Menage a trois: regulation of host immunity by enteric neuro-immune-microbiota cross talks. *Curr Opin Neurobiol.* 2019;62:26-33.
58. Diviccaro S, Giatti S, Borgo F, et al. Steroidogenic machinery in the adult rat colon. *J Steroid Biochem Mol Biol.* 2020;203:105732.
59. Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA. Neurosteroid modulation of GABAA receptors. *Prog Neurobiol.* 2003;71(1):67-80.
60. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci.* 2005;6(7):565-575.
61. Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABAA receptor. *Brain Res.* 1991;561(1):157-161.
62. Wang M, He Y, Eisenman LN, et al. 3beta -hydroxypregnane steroids are pregnenolone sulfate-like GABA(A) receptor antagonists. *J Neurosci.* 2002;22(9):3366-3375.
63. Backstrom T, Wahlstrom G, Wahlstrom K, Zhu D, Wang MD. Isoallopregnanolone; an antagonist to the anaesthetic effect of allopregnanolone in male rats. *Eur J Pharmacol.* 2005;512(1):15-21.
64. Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev.* 2008;60(3):243-260.
65. Whiting PJ. GABA-A receptor subtypes in the brain: a paradigm for CNS drug discovery? *Drug Discov Today.* 2003;8(10):445-450.
66. Sieghart W. Allosteric modulation of GABAA receptors via multiple drug-binding sites. *Adv Pharmacol.* 2015;72:53-96.
67. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology.* 2002;43(4):651-661.
68. Bianchi MT, Macdonald RL. Neurosteroids shift partial agonist activation of GABA(A) receptor channels from low- to high-efficacy gating patterns. *J Neurosci.* 2003;23(34):10934-10943.
69. Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABAA receptors. *Proc Natl Acad Sci USA.* 2003;100(24):14439-14444.
70. Abramian AM, Comenencia-Ortiz E, Modgil A, et al. Neurosteroids promote phosphorylation and membrane insertion of extrasynaptic GABAA receptors. *Proc Natl Acad Sci USA.* 2014;111(19):7132-7137.
71. Adams JM, Thomas P, Smart TG. Modulation of neurosteroid potentiation by protein kinases at synaptic- and extrasynaptic-type GABAA receptors. *Neuropharmacology.* 2015;88:63-73.
72. Gravielle MC. Regulation of GABAA receptors by prolonged exposure to endogenous and exogenous ligands. *Neurochem Int.* 2018;118:96-104.
73. Castellano D, Shepard RD, Lu W. Looking for Novelty in an "Old" Receptor: Recent Advances Toward Our Understanding of GABAARs and Their Implications in Receptor Pharmacology. *Front Neurosci.* 2020;14:616298.
74. Has ATC, Chebib M. GABAA receptors: various stoichiometries of subunit arrangement in alpha1beta3 and alpha1beta3epsilon receptors. *Curr Pharm Des.* 2018;24(17):1839-1844.
75. Caruso D, Pesaresi M, Abbiati F, et al. Comparison of plasma and cerebrospinal fluid levels of neuroactive steroids with their brain, spinal cord and peripheral nerve levels in male and female rats. *Psychoneuroendocrinology.* 2013;38(10):2278-2290.
76. Meffre D, Pianos A, Liere P, et al. Steroid profiling in brain and plasma of male and pseudopregnant female rats after traumatic brain injury: analysis by gas chromatography/mass spectrometry. *Endocrinology.* 2007;148(5):2505-2517.
77. Cohen RE, Wade J. Distribution of two isozymes of 5alpha-reductase in the brains of adult male and female green anole lizards. *Brain Behav Evol.* 2010;76(3-4):279-288.
78. Caruso D, Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Melcangi RC. Effects of short- and long-term gonadectomy on neuroactive steroid levels in the central and peripheral nervous system of male and female rats. *J Neuroendocrinol.* 2010;22:1137-1147.
79. Darbra S, Modol L, Llido A, Casas C, Vallee M, Pallares M. Neonatal allopregnanolone levels alteration: effects on behavior and role of the hippocampus. *Prog Neurobiol.* 2014;113:95-105.
80. Grobin AC, Morrow AL. 3Alpha-hydroxy-5alpha-pregnan-20-one levels and GABA(A) receptor-mediated ³⁶Cl(-) flux across development in rat cerebral cortex. *Brain Res Dev Brain Res.* 2001;131(1-2):31-39.
81. Modol L, Darbra S, Vallee M, Pallares M. Alteration of neonatal Allopregnanolone levels affects exploration, anxiety, aversive

- learning and adult behavioural response to intrahippocampal neurosteroids. *Behav Brain Res*. 2013;241:96-104.
82. Osborne LM, Gispen F, Sanyal A, Yenokyan G, Meilman S, Payne JL. Lower allopregnanolone during pregnancy predicts postpartum depression: an exploratory study. *Psychoneuroendocrinology*. 2017;79:116-121.
 83. Dong E, Matsumoto K, Uzunova V, et al. Brain 5 α -dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. *Proc Natl Acad Sci USA*. 2001;98(5):2849-2854.
 84. Bali A, Jaggi AS. Multifunctional aspects of allopregnanolone in stress and related disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014;48:64-78.
 85. Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5 α -dihydroprogesterone in psychiatric disorders. *Brain Res Brain Res Rev*. 2001;37(1-3):110-115.
 86. Frye CA, Koonce CJ, Edinger KL, Osborne DM, Walf AA. Androgens with activity at estrogen receptor beta have anxiolytic and cognitive-enhancing effects in male rats and mice. *Horm Behav*. 2008;54(5):726-734.
 87. Schule C, Nothdurfter C, Rupprecht R. The role of allopregnanolone in depression and anxiety. *Prog Neurobiol*. 2014;113:79-87.
 88. Rupprecht R, Papadopoulos V, Rammes G, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov*. 2010;9(12):971-988.
 89. Walf AA, Frye CA. Gestational or acute restraint in adulthood reduces levels of 5 α -reduced testosterone metabolites in the hippocampus and produces behavioral inhibition of adult male rats. *Front Cell Neurosci*. 2012;6:40.
 90. Maguire J. Neuroactive steroids and GABAergic involvement in the neuroendocrine dysfunction associated with major depressive disorder and postpartum depression. *Front Cell Neurosci*. 2019;13:83.
 91. Rupprecht R, Holsboer F. Neuropsychopharmacological properties of neuroactive steroids. *Steroids*. 1999;64(1-2):83-91.
 92. Romeo E, Strohle A, Spalletta G, et al. Effects of antidepressant treatment on neuroactive steroids in major depression. *Am J Psychiatry*. 1998;155(7):910-913.
 93. Dichtel LE, Lawson EA, Schorr M, et al. Neuroactive steroids and affective symptoms in women across the weight spectrum. *Neuropsychopharmacology*. 2018;43(6):1436-1444.
 94. Agis-Balboa RC, Guidotti A, Pinna G. 5 α -reductase type I expression is downregulated in the prefrontal cortex/Brodman's area 9 (BA9) of depressed patients. *Psychopharmacology*. 2014;231(17):3569-3580.
 95. Romeo E, Brancati A, De Lorenzo A, et al. Marked decrease of plasma neuroactive steroids during alcohol withdrawal. *Clin Neuropharmacol*. 1996;19(4):366-369.
 96. Jensen JP, Nipper MA, Helms ML, et al. Ethanol withdrawal-induced dysregulation of neurosteroid levels in plasma, cortex, and hippocampus in genetic animal models of high and low withdrawal. *Psychopharmacology*. 2017;234(18):2793-2811.
 97. Milivojevic V, Kranzler HR, Gelernter J, Burian L, Covault J. Variation in genes encoding the neuroactive steroid synthetic enzymes 5 α -reductase type 1 and 3 α -reductase type 2 is associated with alcohol dependence. *Alcohol Clin Exp Res*. 2011;35(5):946-952.
 98. Hasirci AS, Maldonado-Devincci AM, Beattie MC, O'Buckley TK, Morrow AL. Cellular GABAergic neuroactive steroid (3 α ,5 α)-3-hydroxy-pregnan-20-One (3 α ,5 α -THP) immunostaining levels are increased in the ventral tegmental area of human alcohol use disorder patients: a postmortem study. *Alcohol Clin Exp Res*. 2017;41(2):299-311.
 99. Fombonne E. The epidemiology of autism: a review. *Psychol Med*. 1999;29(4):769-786.
 100. Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord*. 2003;33(4):365-382.
 101. Hankin BL, Abramson LY. Development of gender differences in depression: description and possible explanations. *Ann Med*. 1999;31(6):372-379.
 102. Foot M, Koszycki D. Gender differences in anxiety-related traits in patients with panic disorder. *Depress Anxiety*. 2004;20(3):123-130.
 103. Afifi M. Gender differences in mental health. *Singapore Med J*. 2007;48(5):385-391.
 104. Kaye W. Neurobiology of anorexia and bulimia nervosa. *Physiol Behav*. 2008;94(1):121-135.
 105. Mottron L, Duret P, Mueller S, et al. Sex differences in brain plasticity: a new hypothesis for sex ratio bias in autism. *Mol Autism*. 2015;6:33.
 106. Schneider T, Roman A, Basta-Kaim A, et al. Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology*. 2008;33(6):728-740.
 107. Hadj-Sahraoui N, Frederic F, Delhaye-Bouchaud N, Mariani J. Gender effect on Purkinje cell loss in the cerebellum of the heterozygous reeler mouse. *J Neurogenet*. 1996;11(1-2):45-58.
 108. Doulazmi M, Frederic F, Lemaigre-Dubreuil Y, Hadj-Sahraoui N, Delhaye-Bouchaud N, Mariani J. Cerebellar Purkinje cell loss during life span of the heterozygous staggerer mouse (Rora(+)/Rora(sg)) is gender-related. *J Comp Neurol*. 1999;411(2):267-273.
 109. Riecher-Rossler A, Hafner H. Gender aspects in schizophrenia: bridging the border between social and biological psychiatry. *Acta Psychiatr Scand Suppl*. 2000;407:58-62.
 110. Rao ML, Kolsch H. Effects of estrogen on brain development and neuroprotection—implications for negative symptoms in schizophrenia. *Psychoneuroendocrinology*. 2003;28(Suppl 2):83-96.
 111. Hafner H. Gender differences in schizophrenia. *Psychoneuroendocrinology*. 2003;28(Suppl 2):17-54.
 112. Halbreich U, Kahn LS. Hormonal aspects of schizophrenia: an overview. *Psychoneuroendocrinology*. 2003;28(Suppl 2):1-16.
 113. Moriarty PJ, Lieber D, Bennett A, et al. Gender differences in poor outcome patients with lifelong schizophrenia. *Schizophr Bull*. 2001;27(1):103-113.
 114. Seidman LJ, Goldstein JM, Goodman JM, et al. Sex differences in olfactory identification and Wisconsin Card Sorting performance in schizophrenia: relationship to attention and verbal ability. *Biol Psychiatry*. 1997;42(2):104-115.
 115. Goldstein JM, Seidman LJ, Goodman JM, et al. Are there sex differences in neuropsychological functions among patients with schizophrenia? *Am J Psychiatry*. 1998;155(10):1358-1364.
 116. Goldstein JM. Sex, hormones and affective arousal circuitry dysfunction in schizophrenia. *Horm Behav*. 2006;50(4):612-622.
 117. Andreasen NC, Ehrhardt JC, Swayze VW 2nd, et al. Magnetic resonance imaging of the brain in schizophrenia. The pathophysiological significance of structural abnormalities. *Arch Gen Psychiatry*. 1990;47(1):35-44.
 118. Andreasen NC, Flashman L, Flaum M, et al. Regional brain abnormalities in schizophrenia measured with magnetic resonance imaging. *JAMA*. 1994;272(22):1763-1769.
 119. Reite M, Sheeder J, Teale P, et al. Magnetic source imaging evidence of sex differences in cerebral lateralization in schizophrenia. *Arch Gen Psychiatry*. 1997;54(5):433-440.
 120. Bryant NL, Buchanan RW, Vadar K, Breier A, Rothman M. Gender differences in temporal lobe structures of patients with schizophrenia: a volumetric MRI study. *Am J Psychiatry*. 1999;156(4):603-609.
 121. Goldstein JM, Seidman LJ, O'Brien LM, et al. Impact of normal sexual dimorphisms on sex differences in structural brain

- abnormalities in schizophrenia assessed by magnetic resonance imaging. *Arch Gen Psychiatry*. 2002;59(2):154-164.
122. Takahashi T, Suzuki M, Kawasaki Y, et al. Perigenual cingulate gyrus volume in patients with schizophrenia: a magnetic resonance imaging study. *Biol Psychiatry*. 2003;53(7):593-600.
 123. Gur RE, Kohler C, Turetsky BI, et al. A sexually dimorphic ratio of orbitofrontal to amygdala volume is altered in schizophrenia. *Biol Psychiatry*. 2004;55(5):512-517.
 124. Goldstein JM, Seidman LJ, Makris N, et al. Hypothalamic abnormalities in schizophrenia: sex effects and genetic vulnerability. *Biol Psychiatry*. 2007;61(8):935-945.
 125. Pariante CM, Vassilopoulou K, Velakoulis D, et al. Pituitary volume in psychosis. *Br J Psychiatry*. 2004;185:5-10.
 126. Ebihara K, Fujiwara H, Awale S, et al. Decrease in endogenous brain allopregnanolone induces autism spectrum disorder (ASD)-like behavior in mice: A novel animal model of ASD. *Behav Brain Res*. 2017;334:6-15.
 127. Chew L, Sun KL, Sun W, et al. Association of serum allopregnanolone with restricted and repetitive behaviors in adult males with autism. *Psychoneuroendocrinology*. 2021;123:105039.
 128. Pinna G. Animal models of PTSD: the socially isolated mouse and the biomarker role of allopregnanolone. *Front Behav Neurosci*. 2019;13:114.
 129. Almeida FB, Barros HMT, Pinna G. Neurosteroids and neurotrophic factors: what is their promise as biomarkers for major depression and PTSD? *Int J Mol Sci*. 2021;22(4):1758.
 130. Rasmusson AM, Pinna G, Paliwal P, et al. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. *Biol Psychiatry*. 2006;60(7):704-713.
 131. Cruz DA, Glantz LA, McGaughey KD, et al. Neurosteroid levels in the orbital frontal cortex of subjects with PTSD and controls: a preliminary report. *Chronic Stress (Thousand Oaks)*. 2019;3:247054701983857.
 132. Finn DA, Beadles-Bohling AS, Beckley EH, et al. A new look at the 5 α -reductase inhibitor finasteride. *CNS Drug Rev*. 2006;12(1):53-76.
 133. Traish AM, Melcangi RC, Bortolato M, Garcia-Segura LM, Zitzmann M. Adverse effects of 5 α -reductase inhibitors: what do we know, don't know, and need to know? *Rev Endocr Metab Disord*. 2015;16:177-198.
 134. Kaufman KD, Olsen EA, Whiting D, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group. *J Am Acad Dermatol*. 1998;39(4 Pt 1):578-589.
 135. Diviccaro S, Melcangi RC, Giatti S. Post-finasteride syndrome: an emerging clinical problem. *Neurobiol Stress*. 2020;12:100209.
 136. Melcangi RC, Santi D, Spezzano R, et al. Neuroactive steroid levels and psychiatric and andrological features in post-finasteride patients. *J Steroid Biochem Mol Biol*. 2017;171:229-235.
 137. Giatti S, Foglio B, Romano S, et al. Effects of subchronic finasteride treatment and withdrawal on neuroactive steroid levels and their receptors in the male rat brain. *Neuroendocrinology*. 2016;103(6):746-757.
 138. Andersen K, Launer LJ, Dewey ME, et al. Gender differences in the incidence of AD and vascular dementia: the EURODEM Studies. EURODEM Incidence Research Group. *Neurology*. 1999;53(9):1992-1997.
 139. Fratiglioni L, Viitanen M, von Strauss E, Tontodonati V, Herlitz A, Winblad B. Very old women at highest risk of dementia and Alzheimer's disease: incidence data from the Kungsholmen Project. Stockholm. *Neurology*. 1997;48(1):132-138.
 140. Farace E, Alves WM. Do women fare worse: a metaanalysis of gender differences in traumatic brain injury outcome. *J Neurosurg*. 2000;93(4):539-545.
 141. Niemeier JP, Marwitz JH, Leshner K, Walker WC, Bushnik T. Gender differences in executive functions following traumatic brain injury. *Neuropsychol Rehabil*. 2007;17(3):293-313.
 142. Marcus SM, Kerber KB, Rush AJ, et al. Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the Sequenced Treatment Alternatives to Relieve Depression study. *Compr Psychiatry*. 2008;49(3):238-246.
 143. Simonds VM, Whiffen VE. Are gender differences in depression explained by gender differences in co-morbid anxiety? *J Affect Disord*. 2003;77(3):197-202.
 144. Hempel R, Onopa R, Convit A. Type 2 diabetes affects hippocampus volume differentially in men and women. *Diabetes Metab Res Rev*. 2012;28(1):76-83.
 145. Luchetti S, Bossers K, Frajese GV, Swaab DF. Neurosteroid biosynthetic pathway changes in substantia nigra and caudate nucleus in Parkinson's disease. *Brain Pathol*. 2010;20(5):945-951.
 146. Litim N, Morissette M, Caruso D, Melcangi RC, Di Paolo T. Effect of the 5 α -reductase enzyme inhibitor dutasteride in the brain of intact and parkinsonian mice. *J Steroid Biochem Mol Biol*. 2017;174:242-256.
 147. Litim N, Bourque M, Al Sweidi S, Morissette M, Di Paolo T. The 5 α -reductase inhibitor Dutasteride but not Finasteride protects dopamine neurons in the MPTP mouse model of Parkinson's disease. *Neuropharmacology*. 2015;97:86-94.
 148. Caruso D, Melis M, Fenu G, et al. Neuroactive steroid levels in plasma and cerebrospinal fluid of male multiple sclerosis patients. *J Neurochem*. 2014;130(4):591-597.
 149. Noorbakhsh F, Ellestad KK, Maingot F, et al. Impaired neurosteroid synthesis in multiple sclerosis. *Brain*. 2011;134(Pt 9):2703-2721.
 150. Giatti S, D'Intino G, Maschi O, et al. Acute experimental autoimmune encephalomyelitis induces sex dimorphic changes in neuroactive steroid levels. *Neurochem Int*. 2010;56:118-127.
 151. Caruso D, D'Intino G, Giatti S, et al. Sex-dimorphic changes in neuroactive steroid levels after chronic experimental autoimmune encephalomyelitis. *J Neurochem*. 2010;114(3):921-932.
 152. Orefice N, Carotenuto A, Mangone G, et al. Assessment of neuroactive steroids in cerebrospinal fluid comparing acute relapse and stable disease in relapsing-remitting multiple sclerosis. *J Steroid Biochem Mol Biol*. 2016;159:1-7.
 153. Lopez-Rodriguez AB, Acaz-Fonseca E, Giatti S, et al. Correlation of brain levels of progesterone and dehydroepiandrosterone with neurological recovery after traumatic brain injury in female mice. *Psychoneuroendocrinology*. 2015;56:1-11.
 154. Lopez-Rodriguez AB, Acaz-Fonseca E, Spezzano R, et al. Profiling neuroactive steroid levels after traumatic brain injury in male mice. *Endocrinology*. 2016;157(10):3983-3993.
 155. Pesaresi M, Maschi O, Giatti S, Garcia-Segura LM, Caruso D, Melcangi RC. Sex differences in neuroactive steroid levels in the nervous system of diabetic and non-diabetic rats. *Horm Behav*. 2010;57(1):46-55.
 156. Romano S, Mitro N, Diviccaro S, et al. Short-term effects of diabetes on neurosteroidogenesis in the rat hippocampus. *J Steroid Biochem Mol Biol*. 2017;167:135-143.
 157. Romano S, Mitro N, Giatti S, et al. Diabetes induces mitochondrial dysfunction and alters cholesterol homeostasis and neurosteroidogenesis in the rat cerebral cortex. *J Steroid Biochem Mol Biol*. 2018;178:108-116.
 158. Pesaresi M, Giatti S, Spezzano R, et al. Axonal transport in a peripheral diabetic neuropathy model: sex-dimorphic features. *Biol Sex Differ*. 2018;9(1):6.
 159. Cermenati G, Audano M, Giatti S, et al. Lack of sterol regulatory element binding factor-1c imposes glial Fatty Acid utilization leading to peripheral neuropathy. *Cell Metab*. 2015;21(4):571-583.
 160. Mitro N, Cermenati G, Audano M, et al. Sterol regulatory element binding protein-1C knockout mice show altered neuroactive steroid levels in sciatic nerve. *J Neurochem*. 2017;142(3):420-428.
 161. Roglio I, Bianchi R, Gotti S, et al. Neuroprotective effects of dihydroprogesterone and progesterone in an experimental model of nerve crush injury. *Neuroscience*. 2008;155(3):673-685.

162. Gonzalez SL, Meyer L, Raggio MC, et al. Allopregnanolone and progesterone in experimental neuropathic pain: former and new insights with a translational perspective. *Cell Mol Neurobiol*. 2019;39(4):523-537.
163. Patte-Mensah C, Meyer L, Schaeffer V, Mensah-Nyagan AG. Selective regulation of 3 α -hydroxysteroid oxidoreductase expression in dorsal root ganglion neurons: a possible mechanism to cope with peripheral nerve injury-induced chronic pain. *Pain*. 2010;150(3):522-534.
164. Patte-Mensah C, Meyer L, Taleb O, Mensah-Nyagan AG. Potential role of allopregnanolone for a safe and effective therapy of neuropathic pain. *Prog Neurobiol*. 2014;113:70-78.
165. Zhang M, Liu J, Zhou MM, et al. Elevated neurosteroids in the lateral thalamus relieve neuropathic pain in rats with spared nerve injury. *Neurosci Bull*. 2016;32(4):311-322.
166. Frye CA, Sumida K, Lydon JP, O'Malley BW, Pfaff DW. Mid-aged and aged wild-type and progesterone receptor knockout (PRKO) mice demonstrate rapid progesterone and 3 α ,5 α -THP-facilitated lordosis. *Psychopharmacology*. 2006;185(4):423-432.
167. Frye CA, Paris JJ, Rhodes ME. Exploratory, anti-anxiety, social, and sexual behaviors of rats in behavioral estrus is attenuated with inhibition of 3 α ,5 α -THP formation in the midbrain ventral tegmental area. *Behav Brain Res*. 2008;193(2):269-276.
168. Frye CA. Novel substrates for, and sources of, progestogens for reproduction. *J Neuroendocrinol*. 2011;23(11):961-973.
169. Beyer C, Gonzalez-Flores O, Gonzalez-Mariscal G. Ring A reduced progestins potently stimulate estrous behavior in rats: paradoxical effect through the progesterone receptor. *Physiol Behav*. 1995;58(5):985-993.
170. Gonzalez-Mariscal G, Gonzalez-Flores O, Beyer C. Intrahypothalamic injection of RU486 antagonizes the lordosis induced by ring A-reduced progestins. *Physiol Behav*. 1989;46(3):435-438.
171. Belyaeva OV, Chetyrkin SV, Clark AL, et al. Role of microsomal retinol/sterol dehydrogenase-like short-chain dehydrogenases/reductases in the oxidation and epimerization of 3 α -hydroxysteroids in human tissues. *Endocrinology*. 2007;148(5):2148-2156.
172. Chetyrkin SV, Belyaeva OV, Gough WH, Kedishvili NY. Characterization of a novel type of human microsomal 3 α -hydroxysteroid dehydrogenase: unique tissue distribution and catalytic properties. *J Biol Chem*. 2001;276(25):22278-22286.
173. Penning TM. Human hydroxysteroid dehydrogenases and pre-receptor regulation: insights into inhibitor design and evaluation. *J Steroid Biochem Mol Biol*. 2011;125(1-2):46-56.
174. Nicol MB, Hirst JJ, Walker DW. Effect of pregnane steroids on electrocortical activity and somatosensory evoked potentials in fetal sheep. *Neurosci Lett*. 1998;253(2):111-114.
175. Griffin LD, Gong W, Verot L, Mellon SH. Niemann-Pick type C disease involves disrupted neurosteroidogenesis and responds to allopregnanolone. *Nat Med*. 2004;10(7):704-711.
176. Grobin AC, Gizerian S, Lieberman JA, Morrow AL. Perinatal allopregnanolone influences prefrontal cortex structure, connectivity and behavior in adult rats. *Neuroscience*. 2006;138(3):809-819.
177. Cooper EJ, Johnston GA, Edwards FA. Effects of a naturally occurring neurosteroid on GABA_A IPSCs during development in rat hippocampal or cerebellar slices. *J Physiol*. 1999;521(Pt 2):437-449.
178. Bicikova M, Klak J, Hill M, Zizka Z, Hampl R, Calda P. Two neuroactive steroids in midpregnancy as measured in maternal and fetal sera and in amniotic fluid. *Steroids*. 2002;67(5):399-402.
179. Concas A, Mostallino MC, Porcu P, et al. Role of brain allopregnanolone in the plasticity of gamma-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc Natl Acad Sci USA*. 1998;95(22):13284-13289.
180. Brunton PJ, McKay AJ, Ochedalski T, et al. Central opioid inhibition of neuroendocrine stress responses in pregnancy in the rat is induced by the neurosteroid allopregnanolone. *J Neurosci*. 2009;29(20):6449-6460.
181. Brunton PJ, Meddle SL, Ma S, Ochedalski T, Douglas AJ, Russell JA. Endogenous opioids and attenuated hypothalamic-pituitary-adrenal axis responses to immune challenge in pregnant rats. *J Neurosci*. 2005;25(21):5117-5126.
182. Ma S, Shipston MJ, Morilak D, Russell JA. Reduced hypothalamic vasopressin secretion underlies attenuated adrenocorticotropin stress responses in pregnant rats. *Endocrinology*. 2005;146(3):1626-1637.
183. Neumann ID, Johnstone HA, Hatzinger M, et al. Attenuated neuroendocrine responses to emotional and physical stressors in pregnant rats involve adenohipophysial changes. *J Physiol*. 1998;508(Pt 1):289-300.
184. Guerra-Araiza C, Miranda-Martinez A, Neri-Gomez T, Camacho-Arroyo I. Sex steroids effects on the content of GAD, TH, GABA(A), and glutamate receptors in the olfactory bulb of the male rat. *Neurochem Res*. 2008;33(8):1568-1573.
185. Guerra-Araiza C, Amorim MA, Camacho-Arroyo I, Garcia-Segura LM. Effects of progesterone and its reduced metabolites, dihydroprogesterone and tetrahydroprogesterone, on the expression and phosphorylation of glycogen synthase kinase-3 and the microtubule-associated protein tau in the rat cerebellum. *Dev Neurobiol*. 2007;67(4):510-520.
186. Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci USA*. 1991;88(10):4553-4557.
187. Mosher LJ, Cadeddu R, Yen S, et al. Allopregnanolone is required for prepulse inhibition deficits induced by D1 dopamine receptor activation. *Psychoneuroendocrinology*. 2019;108:53-61.
188. Cabrera RJ, Bregonzio C, Laconi M, Mampel A. Allopregnanolone increase in striatal N-methyl-D-aspartic acid evoked [3H]dopamine release is estrogen and progesterone dependent. *Cell Mol Neurobiol*. 2002;22(4):445-454.
189. Khisti RT, Deshpande LS, Chopde CT. The neurosteroid 3 α -hydroxy-5 α -pregnan-20-one affects dopamine-mediated behavior in rodents. *Psychopharmacology*. 2002;161(2):120-128.
190. Wang JM. Allopregnanolone and neurogenesis in the nigrostriatal tract. *Front Cell Neurosci*. 2014;8:224.
191. Bortolato M, Devoto P, Roncada P, et al. Isolation rearing-induced reduction of brain 5 α -reductase expression: relevance to dopaminergic impairments. *Neuropharmacology*. 2011;60(7-8):1301-1308.
192. Dazzi L, Serra M, Vacca G, et al. Depletion of cortical allopregnanolone potentiates stress-induced increase in cortical dopamine output. *Brain Res*. 2002;932(1-2):135-139.
193. Motzo C, Porceddu ML, Maira G, et al. Inhibition of basal and stress-induced dopamine release in the cerebral cortex and nucleus accumbens of freely moving rats by the neurosteroid allopregnanolone. *J Psychopharmacol (Oxf, Engl)*. 1996;10(4):266-272.
194. Laconi MR, Reggiani PC, Penissi A, Yunes R, Cabrera RJ. Allopregnanolone modulates striatal dopaminergic activity of rats under different gonadal hormones conditions. *Neurol Res*. 2007;29(6):622-627.
195. Dornellas APS, Macedo GC, McFarland MH, et al. Allopregnanolone Decreases Evoked Dopamine Release Differently in Rats by Sex and Estrous Stage. *Front Pharmacol*. 2020;11:608887.
196. Misztal T, Kowalczyk P, Mlotkowska P, Marciniak E. The effect of allopregnanolone on enzymatic activity of the DNA base excision repair pathway in the sheep hippocampus and amygdala under natural and stressful conditions. *Int J Mol Sci*. 2020;21(20):7762.
197. Magnaghi V, Parducz A, Frasca A, et al. GABA synthesis in Schwann cells is induced by the neuroactive steroid allopregnanolone. *J Neurochem*. 2010;112(4):980-990.

198. Perego C, Cairano ES, Ballabio M, Magnaghi V. Neurosteroid allopregnanolone regulates EAAC1-mediated glutamate uptake and triggers actin changes in Schwann cells. *J Cell Physiol.* 2011;227:1740-1751.
199. Magnaghi V, Ballabio M, Roglio I, Melcangi RC. Progesterone derivatives increase expression of Krox-20 and Sox-10 in rat Schwann cells. *J Mol Neurosci.* 2007;31(2):149-157.
200. Melcangi RC, Magnaghi V, Cavarretta I, et al. Progesterone derivatives are able to influence peripheral myelin protein 22 and PO gene expression: possible mechanisms of action. *J Neurosci Res.* 1999;56(4):349-357.
201. Melcangi RC, Cavarretta IT, Ballabio M, et al. Peripheral nerves: a target for the action of neuroactive steroids. *Brain Res Brain Res Rev.* 2005;48(2):328-338.
202. Magnaghi V, Cavarretta I, Galbiati M, Martini L, Melcangi RC. Neuroactive steroids and peripheral myelin proteins. *Brain Res Brain Res Rev.* 2001;37(1-3):360-371.
203. Magnaghi V, Veiga S, Ballabio M, Gonzalez LC, Garcia-Segura LM, Melcangi RC. Sex-dimorphic effects of progesterone and its reduced metabolites on gene expression of myelin proteins by rat Schwann cells. *J Peripher Nerv Syst.* 2006;11(2):111-118.
204. Barbaccia ML, Serra M, Purdy RH, Biggio G. Stress and neuroactive steroids. *Int Rev Neurobiol.* 2001;46:243-272.
205. Zorumski CF, Paul SM, Covey DF, Mennerick S. Neurosteroids as novel antidepressants and anxiolytics: GABA-A receptors and beyond. *Neurobiol Stress.* 2019;11:100196.
206. Reddy DS, O'Malley BW, Rogawski MA. Anxiolytic activity of progesterone in progesterone receptor knockout mice. *Neuropharmacology.* 2005;48(1):14-24.
207. Herman JP, Mueller NK, Figueiredo H. Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Ann N Y Acad Sci.* 2004;1018:35-45.
208. Sarkar J, Wakefield S, MacKenzie G, Moss SJ, Maguire J. Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive GABAA receptors. *J Neurosci.* 2011;31(50):18198-18210.
209. Budziszewska B, Zajac A, Basta-Kaim A, et al. Effects of neurosteroids on the human corticotropin-releasing hormone gene. *Pharmacol Rep.* 2010;62(6):1030-1040.
210. Misztal T, Mlotkowska P, Marciniak E, Misztal A. Allopregnanolone reduces neuroendocrine response to acute stressful stimuli in sheep. *J Endocrinol.* 2020;244(1):201-211.
211. D'Aquila PS, Canu S, Sardella M, Spanu C, Serra G, Franconi F. Dopamine is involved in the antidepressant-like effect of allopregnanolone in the forced swimming test in female rats. *Behav Pharmacol.* 2010;21(1):21-28.
212. Nangaku M, Yoshino K, Oda Y, et al. Astroglial glutamate transporter 1 and glutamine synthetase of the nucleus accumbens are involved in the antidepressant-like effects of allopregnanolone in learned helplessness rats. *Behav Brain Res.* 2021;401:113092.
213. Strohle A, Romeo E, Hermann B, et al. Concentrations of 3 alpha-reduced neuroactive steroids and their precursors in plasma of patients with major depression and after clinical recovery. *Biol Psychiatry.* 1999;45(3):274-277.
214. Fry JP, Li KY, Devall AJ, Cockcroft S, Honour JW, Lovick TA. Fluoxetine elevates allopregnanolone in female rat brain but inhibits a steroid microsomal dehydrogenase rather than activating an aldo-keto reductase. *Br J Pharmacol.* 2014;171(24):5870-5880.
215. Brunton PJ, Donadio MV, Yao ST, et al. 5alpha-Reduced neurosteroids sex-dependently reverse central prenatal programming of neuroendocrine stress responses in rats. *J Neurosci.* 2015;35(2):666-677.
216. Anker JJ, Carroll ME. Sex differences in the effects of allopregnanolone on yohimbine-induced reinstatement of cocaine seeking in rats. *Drug Alcohol Depend.* 2010;107(2-3):264-267.
217. Patchev VK, Shoaib M, Holsboer F, Almeida OF. The neurosteroid tetrahydroprogesterone counteracts corticotropin-releasing hormone-induced anxiety and alters the release and gene expression of corticotropin-releasing hormone in the rat hypothalamus. *Neuroscience.* 1994;62(1):265-271.
218. Boero G, Tyler RE, Todd CA, et al. (3alpha,5alpha)3-hydroxypregnan-20-one (3alpha,5alpha-THP) regulation of hypothalamic and extrahypothalamic corticotropin releasing factor (CRF): Sexual dimorphism and brain region specificity in Sprague Dawley rats. *Neuropharmacology.* 2021;186:108463.
219. Timby E, Backstrom T, Nyberg S, Stenlund H, Wihlback AN, Bixo M. Women with premenstrual dysphoric disorder have altered sensitivity to allopregnanolone over the menstrual cycle compared to controls-a pilot study. *Psychopharmacology.* 2016;233(11):2109-2117.
220. Bixo M, Johansson M, Timby E, Michalski L, Backstrom T. Effects of GABA active steroids in the female brain with a focus on the premenstrual dysphoric disorder. *J Neuroendocrinol.* 2018;30(2).
221. Backstrom T, Bixo M, Johansson M, et al. Allopregnanolone and mood disorders. *Prog Neurobiol.* 2014;113:88-94.
222. Mosher LJ, Godar SC, Nelson M, Fowler SC, Pinna G, Bortolato M. Allopregnanolone mediates the exacerbation of Tourette-like responses by acute stress in mouse models. *Sci Rep.* 2017;7(1):3348.
223. Cadeddu R, Backstrom T, Floris G, Nordkild P, Segerdahl M, Bortolato M. Isoallopregnanolone reduces tic-like behaviours in the D1CT-7 mouse model of Tourette syndrome. *J Neuroendocrinol.* 2019;32:e12754.
224. Ciriza I, Carrero P, Frye CA, Garcia-Segura LM. Reduced metabolites mediate neuroprotective effects of progesterone in the adult rat hippocampus. The synthetic progestin medroxyprogesterone acetate (Provera) is not neuroprotective. *J Neurobiol.* 2006;66(9):916-928.
225. Belelli D, Bolger MB, Gee KW. Anticonvulsant profile of the progesterone metabolite 5 alpha-pregnan-3 alpha-ol-20-one. *Eur J Pharmacol.* 1989;166(2):325-329.
226. Beckley EH, Fretwell AM, Tanchuck MA, Gililand KR, Crabbe JC, Finn DA. Decreased anticonvulsant efficacy of allopregnanolone during ethanol withdrawal in female withdrawal seizure-prone vs. withdrawal seizure-resistant mice. *Neuropharmacology.* 2008;54(2):365-374.
227. Singh S, Hota D, Prakash A, Khanduja KL, Arora SK, Chakrabarti A. Allopregnanolone, the active metabolite of progesterone protects against neuronal damage in picrotoxin-induced seizure model in mice. *Pharmacol Biochem Behav.* 2010;94(3):416-422.
228. Czlonkowska AI, Krzascik P, Sienkiewicz-Jaroszc H, et al. The effects of neurosteroids on picrotoxin-, bicuculline- and NMDA-induced seizures, and a hypnotic effect of ethanol. *Pharmacol Biochem Behav.* 2000;67(2):345-353.
229. Frye CA, Scalise TJ. Anti-seizure effects of progesterone and 3alpha,5alpha-THP in kainic acid and perforant pathway models of epilepsy. *Psychoneuroendocrinology.* 2000;25(4):407-420.
230. Afrazi S, Esmaeili-Mahani S, Sheibani V, Abbasnejad M. Neurosteroid allopregnanolone attenuates high glucose-induced apoptosis and prevents experimental diabetic neuropathic pain: in vitro and in vivo studies. *J Steroid Biochem Mol Biol.* 2014;139:98-103.
231. Sayeed I, Guo Q, Hoffman SW, Stein DG. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med.* 2006;47(4):381-389.
232. Ardeshiri A, Kelley MH, Korner IP, Hurn PD, Herson PS. Mechanism of progesterone neuroprotection of rat cerebellar Purkinje cells following oxygen-glucose deprivation. *Eur J Neurosci.* 2006;24(9):2567-2574.

233. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma*. 2005;22(1):106-118.
234. Paris JJ, Zou S, Hahn YK, Knapp PE, Hauser KF. 5 α -reduced progestogens ameliorate mood-related behavioral pathology, neurotoxicity, and microgliosis associated with exposure to HIV-1 Tat. *Brain Behav Immun*. 2016;55:202-214.
235. Labombarda F, Ghomari AM, Liere P, De Nicola AF, Schumacher M, Guennoun R. Neuroprotection by steroids after neurotrauma in organotypic spinal cord cultures: a key role for progesterone receptors and steroidal modulators of GABAA receptors. *Neuropharmacology*. 2013;71:46-55.
236. Noorbakhsh F, Baker GB, Power C. Allopregnanolone and neuroinflammation: a focus on multiple sclerosis. *Front Cell Neurosci*. 2014;8:134.
237. Irwin RW, Brinton RD. Allopregnanolone as regenerative therapeutic for Alzheimer's disease: translational development and clinical promise. *Prog Neurobiol*. 2014;113:40-55.
238. Irwin RW, Solinsky CM, Brinton RD. Frontiers in therapeutic development of allopregnanolone for Alzheimer's disease and other neurological disorders. *Front Cell Neurosci*. 2014;8:203.
239. Wang T, Yao J, Chen S, Mao Z, Brinton RD. Allopregnanolone reverses bioenergetic deficits in female triple transgenic Alzheimer's mouse model. *Neurotherapeutics*. 2019;17:178-188.
240. Hernandez GD, Solinsky CM, Mack WJ, et al. Safety, tolerability, and pharmacokinetics of allopregnanolone as a regenerative therapeutic for Alzheimer's disease: a single and multiple ascending dose phase 1b/2a clinical trial. *Alzheimers Dement (NY)*. 2020;6(1):e12107.
241. Zolkowska D, Wu CY, Rogawski MA. Intranasal allopregnanolone confers rapid seizure protection: evidence for direct nose-to-brain delivery. *Neurotherapeutics*. 2021;18:544-555.
242. Levesque M, Biagini G, Avoli M. Neurosteroids and Focal Epileptic Disorders. *Int J Mol Sci*. 2020;21(24).
243. Lucchi C, Costa AM, Rustichelli C, Biagini G. Allopregnanolone and pregnanolone are reduced in the hippocampus of epileptic rats, but only allopregnanolone correlates with the seizure frequency. *Neuroendocrinology*. 2021;111:536-541.
244. Lucchi C, Costa AM, Senn L, Messina S, Rustichelli C, Biagini G. Augmentation of endogenous neurosteroid synthesis alters experimental status epilepticus dynamics. *Epilepsia*. 2020;61(9):e129-e134.
245. Meyer M, Garay LI, Kruse MS, et al. Protective effects of the neurosteroid allopregnanolone in a mouse model of spontaneous motoneuron degeneration. *J Steroid Biochem Mol Biol*. 2017;174:201-216.
246. Nezhadi A, Sheibani V, Esmaeilpour K, Shabani M, Esmaeili-Mahani S. Neurosteroid allopregnanolone attenuates cognitive dysfunctions in 6-OHDA-induced rat model of Parkinson's disease. *Behav Brain Res*. 2016;305:258-264.
247. Adeosun SO, Hou X, Jiao Y, et al. Allopregnanolone reinstates tyrosine hydroxylase immunoreactive neurons and motor performance in an MPTP-lesioned mouse model of Parkinson's disease. *PLoS One*. 2012;7(11):e50040.
248. Wang JY, Trivedi AM, Carrillo NR, et al. Open-label allopregnanolone treatment of men with fragile X-associated tremor/ataxia syndrome. *Neurotherapeutics*. 2017;14(4):1073-1083.
249. Napoli E, Schneider A, Wang JY, et al. Allopregnanolone treatment improves plasma metabolomic profile associated with GABA metabolism in fragile X-associated tremor/ataxia syndrome: a pilot study. *Mol Neurobiol*. 2019;56(5):3702-3713.
250. Ahmad I, Lope-Piedrafita S, Bi X, et al. Allopregnanolone treatment, both as a single injection or repetitively, delays demyelination and enhances survival of Niemann-Pick C mice. *J Neurosci Res*. 2005;82(6):811-821.
251. Liao G, Cheung S, Galeano J, Ji AX, Qin Q, Bi X. Allopregnanolone treatment delays cholesterol accumulation and reduces autophagic/lysosomal dysfunction and inflammation in Npc1^{-/-} mouse brain. *Brain Res*. 2009;1270:140-151.
252. Hovakimyan M, Maass F, Petersen J, et al. Combined therapy with cyclodextrin/allopregnanolone and miglustat improves motor but not cognitive functions in Niemann-Pick Type C1 mice. *Neuroscience*. 2013;252:201-211.
253. Kelley MH, Kuroiwa M, Taguchi N, Herson PS. Sex difference in sensitivity to allopregnanolone neuroprotection in mice correlates with effect on spontaneous inhibitory post synaptic currents. *Neuropharmacology*. 2011;61(4):724-729.
254. Reddy DS, Carver CM, Clossen B, Wu X. Extrasynaptic gamma-aminobutyric acid type A receptor-mediated sex differences in the antiseizure activity of neurosteroids in status epilepticus and complex partial seizures. *Epilepsia*. 2019;60(4):730-743.
255. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell*. 2010;140(6):918-934.
256. Tansey MG, Goldberg MS. Neuroinflammation in Parkinson's disease: its role in neuronal death and implications for therapeutic intervention. *Neurobiol Dis*. 2010;37(3):510-518.
257. Tansey MG. Inflammation in neuropsychiatric disease. *Neurobiol Dis*. 2010;37(3):491-492.
258. Wee YV. Inflammation in neurological disorders: a help or a hindrance? *Neuroscientist*. 2010;16(4):408-420.
259. Wuwongse S, Chang RC, Law AC. The putative neurodegenerative links between depression and Alzheimer's disease. *Prog Neurobiol*. 2010;91(4):362-375.
260. Meyer U, Schwarz MJ, Muller N. Inflammatory processes in schizophrenia: a promising neuroimmunological target for the treatment of negative/cognitive symptoms and beyond. *Pharmacol Ther*. 2011;132(1):96-110.
261. Balan I, Beattie MC, O'Buckley TK, Aurelian L, Morrow AL. Endogenous neurosteroid (3 α ,5 α)3-Hydroxypregnan-20-one inhibits toll-like-4 receptor activation and pro-inflammatory signaling in macrophages and brain. *Sci Rep*. 2019;9(1):1220.
262. Balan I, Aurelian L, Schleicher R, Boero G, O'Buckley T, Morrow AL. Neurosteroid allopregnanolone (3 α ,5 α -THP) inhibits inflammatory signals induced by activated MyD88-dependent toll-like receptors. *Transl Psychiatry*. 2021;11(1):145.
263. Ishrat T, Sayeed I, Atif F, Hua F, Stein DGCP. Progesterone and allopregnanolone attenuate blood-brain barrier dysfunction following permanent focal ischemia by regulating the expression of matrix metalloproteinases. *Exp Neurol*. 2010;226:183-190.
264. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol*. 2004;189(2):404-412.
265. VanLandingham JW, Cekic M, Cutler S, Hoffman SW, Stein DGCP. Neurosteroids reduce inflammation after TBI through CD55 induction. *Neurosci Lett*. 2007;425:94-98.
266. Jolivel V, Brun S, Biname F, et al. Microglial cell morphology and phagocytic activity are critically regulated by the neurosteroid allopregnanolone: a possible role in neuroprotection. *Cells*. 2021;10(3):698.
267. Leonelli E, Bianchi R, Cavaletti G, et al. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. *Neuroscience*. 2007;144(4):1293-1304.
268. Azcoitia I, Leonelli E, Magnaghi V, Veiga S, Garcia-Segura LM, Melcangi RC. Progesterone and its derivatives dihydroprogesterone and tetrahydroprogesterone reduce myelin fiber morphological abnormalities and myelin fiber loss in the sciatic nerve of aged rats. *Neurobiol Aging*. 2003;24(6):853-860.
269. Meyer L, Taleb O, Patte-Mensah C, Mensah-Nyagan AG. Neurosteroids and neuropathic pain management: Basic evidence

- and therapeutic perspectives. *Front Neuroendocrinol.* 2019;55:100795.
270. Patte-Mensah C, Kibaly C, Mensah-Nyagan AG. Substance P inhibits progesterone conversion to neuroactive metabolites in spinal sensory circuit: a potential component of nociception. *Proc Natl Acad Sci USA.* 2005;102(25):9044-9049.
 271. Meyer L, Patte-Mensah C, Taleb O, Mensah-Nyagan AG. Cellular and functional evidence for a protective action of neurosteroids against vincristine chemotherapy-induced painful neuropathy. *Cell Mol Life Sci.* 2010;67(17):3017-3034.
 272. Meyer L, Patte-Mensah C, Taleb O, Mensah-Nyagan AG. Allopregnanolone prevents and suppresses oxaliplatin-evoked painful neuropathy: multi-parametric assessment and direct evidence. *Pain.* 2011;152(1):170-181.
 273. Kawano T, Soga T, Chi H, et al. Role of the neurosteroid allopregnanolone in the hyperalgesic behavior induced by painful nerve injury in rats. *J Anesth.* 2011;25(6):942-945.
 274. Pathirathna S, Brimelow BC, Jagodic MM, et al. New evidence that both T-type calcium channels and GABAA channels are responsible for the potent peripheral analgesic effects of 5 α -reduced neuroactive steroids. *Pain.* 2005;114(3):429-443.
 275. Pathirathna S, Todorovic SM, Covey DF, Jevtovic-Todorovic V. 5 α -reduced neuroactive steroids alleviate thermal and mechanical hyperalgesia in rats with neuropathic pain. *Pain.* 2005;117(3):326-339.
 276. Wirth MM. Beyond the HPA Axis: progesterone-derived neuroactive steroids in human stress and emotion. *Front Endocrinol (Lausanne).* 2011;2:19.
 277. Reddy DS, Estes WA. Clinical potential of neurosteroids for CNS disorders. *Trends Pharmacol Sci.* 2016;37(7):543-561.
 278. Irwin RW, Solinsky CM, Loya CM, et al. Allopregnanolone pre-clinical acute pharmacokinetic and pharmacodynamic studies to predict tolerability and efficacy for Alzheimer's disease. *PLoS One.* 2015;10(6):e0128313.
 279. Schumacher M, Mattern C, Ghomari A, et al. Revisiting the roles of progesterone and allopregnanolone in the nervous system: resurgence of the progesterone receptors. *Prog Neurobiol.* 2014;113:6-39.
 280. Taleb O, Patte-Mensah C, Meyer L, et al. Evidence for effective structure-based neuromodulatory effects of new analogues of neurosteroid allopregnanolone. *J Neuroendocrinol.* 2018;30(2).
 281. Rey M, Coirini H. Synthetic neurosteroids on brain protection. *Neural Regen Res.* 2015;10(1):17-21.
 282. Rey M, Veleiro AS, Ghini AA, Kruse MS, Burton G, Coirini H. Effect of synthetic steroids on GABAA receptor binding in rat brain. *Neuroscience.* 2015;290:138-146.
 283. Carter RB, Wood PL, Wieland S, et al. Characterization of the anti-convulsant properties of ganaxolone (CCD 1042; 3 α -hydroxy-3 β -methyl-5 α -pregnan-20-one), a selective, high-affinity, steroid modulator of the gamma-aminobutyric acid(A) receptor. *J Pharmacol Exp Ther.* 1997;280(3):1284-1295.
 284. Rey M, Kruse MS, Alvarez LD, et al. Neuroprotective action of synthetic steroids with oxygen bridge. Activity on GABAA receptor. *Exp Neurol.* 2013;249:49-58.
 285. Althaus AL, McCarren HS, Alqazzaz A, et al. The synthetic neuroactive steroid SGE-516 reduces status epilepticus and neuronal cell death in a rat model of soman intoxication. *Epilepsy Behav.* 2017;68:22-30.
 286. Karout M, Miesch M, Geoffroy P, et al. Novel analogs of allopregnanolone show improved efficiency and specificity in neuroprotection and stimulation of proliferation. *J Neurochem.* 2016;139(5):782-794.
 287. Mellon SH, Gong W, Schonemann MD. Endogenous and synthetic neurosteroids in treatment of Niemann-Pick Type C disease. *Brain Res Rev.* 2008;57(2):410-420.
 288. Pinna G, Rasmusson AM. Ganaxolone improves behavioral deficits in a mouse model of post-traumatic stress disorder. *Front Cell Neurosci.* 2014;8:256.
 289. Ciarlone SL, Wang X, Rogawski MA, Weeber EJ. Effects of the synthetic neurosteroid ganaxolone on seizure activity and behavioral deficits in an Angelman syndrome mouse model. *Neuropharmacology.* 2017;116:142-150.
 290. Chuang SH, Reddy DS. 3 β -Methyl-Neurosteroid Analogs Are Preferential Positive Allosteric Modulators and Direct Activators of Extrasynaptic delta-Subunit gamma-Aminobutyric Acid Type A Receptors in the Hippocampus Dentate Gyrus Subfield. *J Pharmacol Exp Ther.* 2018;365(3):583-601.
 291. Saporito MS, Gruner JA, DiCamillo A, Hinchliffe R, Barker-Haliski M, White HS. Intravenously administered ganaxolone blocks diazepam-resistant lithium-pilocarpine-induced status epilepticus in rats: comparison with allopregnanolone. *J Pharmacol Exp Ther.* 2019;368(3):326-337.
 292. Zolkowska D, Wu CY, Rogawski MA. Intramuscular allopregnanolone and ganaxolone in a mouse model of treatment-resistant status epilepticus. *Epilepsia.* 2018;59(Suppl 2):220-227.
 293. Shaw JC, Dyson RM, Palliser HK, Gray C, Berry MJ, Hirst JJ. Neurosteroid replacement therapy using the allopregnanolone-analogue ganaxolone following preterm birth in male guinea pigs. *Pediatr Res.* 2019;85(1):86-96.
 294. Paul AM, Branton WG, Walsh JG, et al. GABA transport and neuroinflammation are coupled in multiple sclerosis: regulation of the GABA transporter-2 by ganaxolone. *Neuroscience.* 2014;273:24-38.
 295. Mouihate A, Kalakh S. Ganaxolone enhances microglial clearance activity and promotes remyelination in focal demyelination in the corpus callosum of ovariectomized rats. *CNS Neurosci Ther.* 2020;26:240-250.
 296. Nipper MA, Jensen JP, Helms ML, et al. Genotype differences in sensitivity to the anticonvulsant effect of the synthetic neurosteroid ganaxolone during chronic ethanol withdrawal. *Neuroscience.* 2019;397:127-137.
 297. Meltzer-Brody S, Colquhoun H, Riesenberger R, et al. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet.* 2018;392(10152):1058-1070.
 298. Zheng W, Cai DB, Zheng W, et al. Brexanolone for postpartum depression: A meta-analysis of randomized controlled studies. *Psychiatry Res.* 2019;279:83-89.
 299. Morrison KE, Cole AB, Thompson SM, Bale TL. Brexanolone for the treatment of patients with postpartum depression. *Drugs Today (Barc).* 2019;55(9):537-544.
 300. Cristea IA, Naudet F. US food and drug administration approval of esketamine and brexanolone. *Lancet Psychiatry.* 2019;6(12):975-977.
 301. Papadopoulos V, Amri H, Li H, Boujrad N, Vidic B, Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. *J Biol Chem.* 1997;272(51):32129-32135.
 302. Daugherty DJ, Selvaraj V, Chechneva OV, Liu XB, Pleasure DE, Deng W. A TSPO ligand is protective in a mouse model of multiple sclerosis. *EMBO Mol Med.* 2013;5(6):891-903.
 303. Leva G, Klein C, Benyounes J, et al. The translocator protein ligand XBD173 improves clinical symptoms and neuropathological markers in the SJL/J mouse model of multiple sclerosis. *Biochim Biophys Acta Mol Basis Dis.* 2017;1863(12):3016-3027.
 304. Miao YL, Guo WZ, Shi WZ, et al. Midazolam ameliorates the behavior deficits of a rat posttraumatic stress disorder model through dual 18 kDa translocator protein and central benzodiazepine receptor and neurosteroidogenesis. *PLoS One.* 2014;9(7):e101450.

305. Shang C, Guo Y, Yao JQ, et al. Rapid anti-PTSD-like activity of the TSPO agonist YL-IPA08: emphasis on brain GABA, neurosteroids and HPA axis function. *Behav Brain Res*. 2019;112320.
306. Ishikawa M, Yoshitomi T, Covey DF, Zorumski CF, Izumi Y. TSPO activation modulates the effects of high pressure in a rat ex vivo glaucoma model. *Neuropharmacology*. 2016;111:142-159.
307. Mitro N, Cermenati G, Giatti S, et al. LXR and TSPO as new therapeutic targets to increase the levels of neuroactive steroids in the central nervous system of diabetic animals. *Neurochem Int*. 2012;60(6):616-621.
308. Giatti S, Pesaresi M, Cavaletti G, et al. Neuroprotective effects of a ligand of translocator protein-18kDa (Ro5-4864) in experimental diabetic neuropathy. *Neuroscience*. 2009;164:520-529.
309. Qiu ZK, He JL, Liu X, et al. The antidepressant-like activity of AC-5216, a ligand for 18KDa translocator protein (TSPO), in an animal model of diabetes mellitus. *Sci Rep*. 2016;6:37345.
310. Cermenati G, Giatti S, Cavaletti G, et al. Activation of the liver X receptor increases neuroactive steroid levels and protects from diabetes-induced peripheral neuropathy. *J Neurosci*. 2010;30(36):11896-11901.
311. Baulieu EE, Robel P. Neurosteroids: a new brain function? *J Steroid Biochem Mol Biol*. 1990;37(3):395-403.

How to cite this article: Diviccaro S, Cioffi L, Falvo E, Giatti S, Melcangi RC. Allopregnanolone: An overview on its synthesis and effects. *J Neuroendocrinol*. 2021;00:e12996. <https://doi.org/10.1111/jne.12996>