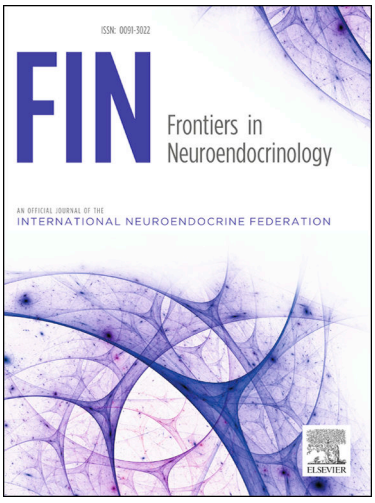
Journal Pre-proofs



The role of neurosteroids in posttraumatic stress disorder and alcohol use disorder: A review of 10 years of clinical literature and treatment implications

MacKenzie R. Peltier, Terril L. Verplaetse, Margaret Altemus, Yasmin Zakiniaeiz, Elizabeth A. Ralevski, Yann S. Mineur, Ralitza Gueorguieva, Marina R. Picciotto, Kelly P. Cosgrove, Ismene Petrakis, Sherry A. McKee

PII: S0091-3022(23)00067-5
DOI: https://doi.org/10.1016/j.yfrne.2023.101119
Reference: YFRNE 101119

To appear in: *Frontiers in Neuroendocrinology*

Received Date: 30 April 2023
Revised Date: 8 December 2023
Accepted Date: 31 December 2023

Please cite this article as: M.R. Peltier, T.L. Verplaetse, M. Altemus, Y. Zakiniaeiz, E.A. Ralevski, Y.S. Mineur, R. Gueorguieva, M.R. Picciotto, K.P. Cosgrove, I. Petrakis, S.A. McKee, The role of neurosteroids in posttraumatic stress disorder and alcohol use disorder: A review of 10 years of clinical literature and treatment implications, *Frontiers in Neuroendocrinology* (2024), doi: https://doi.org/10.1016/j.yfrne.2023.101119

Published by Elsevier Inc.

Words: 11,106 (8,000 to 15,000 words)

Abstract: 150 (150 max)

References: 195

Tables: 3

Figures: 1

The role of neurosteroids in posttraumatic stress disorder and alcohol use disorder: A review of 10 years of clinical literature and treatment implications

MacKenzie R. Peltier, PhD[a,b,c], Terril L. Verplaetse, PhD[a], Margaret Altemus, MD[a,b], Yasmin Zakiniaeiz, PhD[a], Elizabeth A. Ralevski, PhD[a,b], Yann S. Mineur, PhD[a], Ralitza Gueorguieva, PhD[a,d], Marina R. Picciotto, PhD[a], Kelly P. Cosgrove, PhD[a,c,e], Ismene Petrakis, MD[a,b,c], & Sherry A. McKee, PhD[a]

[a] Yale School of Medicine, Department of Psychiatry, New Haven, CT 06519

[b] VA Connecticut Healthcare System, Mental Health Service, West Haven, CT 06516

[c] National Center for PTSD, Clinical Neuroscience Division, West Haven, CT 06516

[d] Department of Biostatistics, School of Public Health, Yale University, New Haven, CT, USA

[e] Department of Radiology and Biomedical Imaging, School of Medicine, Yale University, New Haven, CT, USA

Corresponding Author:

MacKenzie R. Peltier, PhD

Department of Psychiatry

Yale School of Medicine

40 Temple Street

Suite: 5B

New Haven, CT 06519

Email: mackenzie.peltier@yale.edu

Invited Review: Frontiers in Neuroendocrinology

Funding: National Institute of Alcohol Abuse and Alcoholism U54AA027989 (SAM), P01AA02747307 (SAM), K01AA025670 (TLV), R03AA028361 (TLV), K01AA029706 (YZ), R21AA024917 (EAR); National Institute of Drug Abuse R01DA047986 (SAM), R37 DA014241 (MRP); National Institute of Mental Health R01MH110674 (KPC); Veterans' Affairs l01CX001517 (ILP); Veteran' Affairs New England CDA1 Award (MRP)

Declaration of Interests: None

Abstract

Rates of alcohol use disorder (AUD) are increasing in men and women and there are high rates of concurrent posttraumatic stress disorder (PTSD) and AUD. AUD and PTSD synergistically increase symptomatology and negatively affect treatment outcomes; however, there are very limited pharmacological treatments for PTSD/AUD. Neurosteroids have been implicated in the underlying neurobiological mechanisms of both PTSD and AUD and may be a target for treatment development. This review details the past ten years of research on pregnenolone, progesterone, allopregnanolone, pregnanolone, estradiol, testosterone and dehydroepiandrosterone/dehydroepiandrosterone-sulfate (DHEA/DHEA-S) in the context of PTSD and AUD, including examination of trauma/alcohol-related variables, such as stress-reactivity. Emerging evidence that exogenous pregnenolone, progesterone, and allopregnanolone may be promising, novel interventions is also discussed. Specific emphasis is placed on examining the application of sex as a biological variable in this body of literature, given that women are more susceptible to both PTSD diagnoses and stress-related alcohol consumption.

Keywords: neurosteroids, posttraumatic stress disorder, alcohol use disorder, sex as a biological variable

1. Introduction

Alcohol use, including high-risk drinking and alcohol use disorder (AUD) are increasing at alarming rates[1,2] and are identified as the leading risk for disease among individuals 25-49 years old.[3] In 2021, 16.2 million adults in the United States reported heavy drinking in the past month, while 28.6 million adults met diagnostic criteria for AUD in the past year.[4] Posttraumatic Stress Disorder (PTSD) is also highly prevalent among adults. Approximately 70% of adults report exposure to a potentially traumatic event and over six percent of adults in the United States have met diagnostic criteria for PTSD.[5]

There are high rates of psychiatric multimorbidities in the context of AUD,[2,6] including PTSD. Individuals with AUD are more likely to be diagnosed with concurrent PTSD than individuals without AUD.[2] In fact, those having a lifetime diagnosis of AUD are 3 times more likely to also be diagnosed with PTSD.[2] Similarly, individuals diagnosed with PTSD are 1.2-1.7 times more likely to have an AUD compared to individuals without PTSD, indicating that more than 40% of individuals with PTSD also meet diagnostic criteria for an AUD.[5,7]

PTSD and AUD synergistically contribute to more severe symptomatology as part of this reciprocal relationship.[8-10] PTSD is associated with risky alcohol use[11] and alcohol use increases following a traumatic event.[12] The majority of individuals with previous problematic alcohol use, including both men and women, use alcohol to cope with emotions following the traumatic event.[12] Additionally, greater trauma symptom severity has been linked to increased subsequent alcohol use, while greater alcohol use is related to increased severity in future trauma symptoms among individuals completing treatment for PTSD/AUD.[13] Specifically, trauma-related symptoms such as intrusive thoughts and emotional numbing predict greater alcohol use,[14] while AUD has been associated with increased intrusive and avoidance-based symptoms.[9] These data highlight a bidirectional relationship between the two disorders.

The reciprocal relationship between AUD and PTSD is especially problematic as these concurrent disorders are related to increased functional impairment and lower treatment success.[8,11,15,16] Compared to those with either AUD or PTSD alone, individuals with both disorders have higher rates of other psychiatric diagnoses, increased prevalence of suicide attempts, endorse more trauma-related and alcohol-related symptoms, and have an earlier onset of PTSD diagnosis.[11,15] These individuals are also more likely to use substances to cope with symptoms compared to individuals without the other concurrent disorder.[11] Evidence also suggests that PTSD/AUD is related to impaired physical and mental function and overall lower quality of life.[15] While individuals with PTSD/AUD have higher rates of treatment enrollment than those with either disorder alone,[11,16] treatments are plagued by high drop-out rates[17] and symptoms often persist following treatment.[16]

Limited pharmacological treatments are available to target concurrent trauma symptoms and alcohol consumption. Thus, novel treatments are urgently needed for PTSD/AUD. A recent review illustrated that only nine studies to date have explored pharmacotherapies for concurrent PTSD and AUD.[18] Unsurprisingly, medications for the treatment of PTSD (i.e., sertraline, desipramine) can reduce trauma-related symptoms, and medications for AUD (i.e., naltrexone) can affect alcohol outcomes, but no treatment has demonstrated clear efficacy in reducing both trauma symptoms and alcohol use in individuals with concurrent PTSD and AUD.[18] This evidence suggests that the reciprocal relationship between PTSD and AUD is likely not targeted with currently available pharmacotherapies and there is a need to identify novel treatment targets to address the underlying mechanisms of both PTSD and AUD.

The limited, effective treatment options for concurrent PTSD and AUD is especially problematic for women. Women are drinking at increasing rates compared to men[1,19] and are particularly vulnerable to the development of AUD, as well as the use of and relapse to alcohol in the context of stress.[20] Additionally, women are twice as likely to be diagnosed with PTSD following a traumatic event compared to men,[7] despite experiencing fewer “potentially traumatic” events.[21] Women with PTSD are also more likely to drink to cope with trauma symptoms.[22]

Given the increasing rates of alcohol use, drinking to cope with distress, and high rates of PTSD among women, it is especially critical to explore treatment targets for women with PTSD/AUD.

1.2 Current Review

Emerging evidence suggests that pregnenolone-based neurosteroids may be a potential treatment target for PTSD/AUD. Our group has previously outlined the therapeutic potential of neurosteroids (including progesterone, allopregnanolone, pregnanolone, estradiol, testosterone and DHEA) for stress-related psychiatric disorders (i.e., disorders characterized by stress and/or negative affect) and alcohol use.[23] At the time of that review, research had demonstrated that there are altered levels of neurosteroids in stress-related psychiatric disorders and in alcohol use, with differing results among the neurosteroids studied. These data illustrated that neurosteroids likely affect the underlying neurobiological mechanisms of stress-related psychiatric disorders, as well as AUD. However, these associations are not often linear. It is therefore critical to better understand how neurosteroids might address the overlapping vulnerabilities attributed to stress, negative affect, and alcohol use. Nonetheless, existing findings are promising and may offer therapeutic potential for PTSD and AUD.

The current review builds upon this previous work, by exploring the emerging literature over the past ten years detailing the role of pregnenolone-based neurosteroids specific to PTSD and alcohol in human populations. Subsequent sections will provide background information on prominent neurosteroids, including pregnenolone, progesterone, pregnanolone, allopregnanolone, estradiol, testosterone, and dehydroepiandrosterone (-sulfate; DHEA/DHEA-S), and then explore the role of these neurosteroids in the symptomatology of PTSD and alcohol use. These sections will conclude with an overview of current treatment studies using exogenous neurosteroids for the treatment of PTSD and alcohol use, as well as future directions for clinical research. While discussing this literature we will highlight sex differences, including sexually dimorphic findings, to provide a full scope of the application of sex as a biological variable (SABV) and understand the current literature from a rigor and reproducibility standpoint. This information will inform future treatment development, especially for women who are vulnerable to PTSD and stress-related alcohol use.

2. Neurosteroids

Neurosteroids are endogenous neuromodulators defined by rapid non-genomic actions[24,25] synthesized in both the brain and peripheral nervous system, as well as in other tissues.[26,27] Previous studies have explored the role of neurosteroids in brain function as well as their treatment potential for a variety of neurological and psychiatric disorders (for review see[25,27-30]). Based upon the available literature, this review will focus on pregnenolone, progesterone, pregnanolone, allopregnanolone, estradiol, testosterone, and DHEA/DHEA-S (See Figure 1).

Pregnenolone is synthesized from cholesterol in the brain and in a wide variety of peripheral tissues. Circulating pools of pregnenolone and other neurosteroids come from the gonads and adrenal glands due to significantly higher volumes of steroid production.[26,27,31] Metabolism of pregnenolone via multiple pathways result in a cascade of well characterized neurosteroids, including pregnenolone-sulfate (pregnenolone-s), progesterone, allopregnanolone, and pregnanolone, as well as testosterone, estrogens (i.e., estradiol), and DHEA/DHEA-S.[27] These neurosteroids are lipophilic and thus easily cross the blood-brain barrier.[31] However, various active transports and enzymatic conversions can effect peripheral and central levels of neurosteroids. Nonetheless, their accumulation in the brain from *de novo* synthesis or the peripheral circulation has rapid effects on multiple neurotransmitter systems[31] related to development of PTSD and AUD, including γ -Aminobutyric acid (GABA)ergic and glutamatergic systems.[31,32] These pregnenolone-derived neurosteroids are also closely related given the

synthesis pathways (see Figure 1). Despite this, it is important to acknowledge that factors including biosynthetic enzyme blockades, cofactor saturations, or levels or metabolic enzymes may impact such synthesis.

Due to its role as a precursor to the neurosteroids mentioned above, pregnenolone increases circulating levels of neurosteroids, including allopregnanolone.[27,29] Unlike other neurosteroids, it has poor affinity for GABA-A receptors, as well as low bioavailability and rapid metabolism, suggesting its therapeutic potential may be mediated by downstream metabolites.[27] Nonetheless, pregnenolone has been identified as a ligand for sigma1 receptors and type-1 cannabinoid receptors.[27] Pregnenolone has also been implicated as a biomarker in pre-clinical studies for anxiety/depression, cognitive functioning, stress-related disorders, schizophrenia, and drug use,[27,29,33] as well as clinical studies of schizophrenia and schizoaffective disorder.[34]

Progesterone is a 21-carbon hormone that is metabolized from pregnenolone by the 3β-hydroxysteroid dehydrogenase enzyme.[35] Progesterone is subsequently converted into allopregnanolone via a two-step process. Progesterone becomes 5α-dihydroprogesterone (5α-DHP) via 5α-reductase and 5α-DHP is converted into allopregnanolone via 3α-hydroxysteroid-dehydrogenase (3α-HSD).[36] Pregnanolone is derived from progesterone by a similar two-step process. 5β-reductase converts progesterone to 5β-dihydroprogesterone (5β-DHP). 5β-DHP is then converted into pregnanolone via 3α-HSD.[37] Notably, the conversion of dihydroprogesterone via 3α-HSD into either allopregnanolone or pregnanolone is a bidirectional reaction. 5α-DHP or 5β-DHP can be reduced to allopregnanolone or pregnanolone, respectively. Conversely allopregnanolone and pregnanolone can be reconverted into 5α-DHP or 5β-DHP.[38] Progesterone and its metabolites, allopregnanolone and pregnanolone, act through genomic and nongenomic mechanisms.[39] Specifically, progesterone, 5α-DHP, and 5β-DHP have genomic action, whereas allopregnanolone and pregnanolone have non-genomic effects.[38,40] Genomic, or classic steroid action, is a relatively slow process, in which progesterone binds to intracellular progesterone receptors, which in turn regulate genetic transcription. The nongenomic function, or “neurosteroid actions” occur via a more immediate interaction with various neurotransmitter systems, including $GABA_A$, glycine, sigma1, and serotonin receptors.[39,41]

For example, fluctuations in neurosteroid levels have been shown to impact the excitatory and inhibitory signaling via GABAergic mechanisms of action. Generally low concentrations of neurosteroid levels increase excitatory signaling via the potentiation of the extrasynaptic GABA-A receptors, whereas large increases in neurosteroid levels result in reduced excitability and enhance GABAergic inhibition.[42] Such shifts in the balance between excitatory and inhibitory GABAergic signaling are also related to the duration of exposure to neurosteroid levels. Acute, small increases (e.g., following a stressor), result in increased tonic inhibition via preferential action at receptors containing the δ subunit, whereas chronic exposure to high levels of neurosteroids (e.g., pregnancy) result in tonic and phasic inhibition (via receptors containing the γ2 subunit).[43]

In particular, progesterone, allopregnanolone, and pregnanolone have positive modulatory effects on $GABA_A$ receptors, which result in increased GABAergic signaling.[29,39] This increase in GABA transmission may be advantageous in the treatment of stress-related disorders, as well as alcohol use and GABAergic agents are emerging as a promising treatment target for concurrent PTSD and AUD.[32] For example, allopregnanolone is a one of the more potent allosteric modulator of $GABA_A$ receptors.[44] Pretreatment with allopregnanolone decreases stress responses and chronic stress reduces allopregnanolone levels. Allopregnanolone levels also quickly rise during acute stress, and provide negative feedback on the acute stress response.[39]

Allopregnanolone downregulates expression of the corticotropin-releasing hormone (CRH) gene, thereby decreasing activity of the hypothalamic–pituitary–adrenal (HPA) axis, likely resulting in its anxiolytic effects.[45] Additionally, preclinical evidence suggests that after a stress-based exposure, progesterone's metabolites, including allopregnanolone, enhance GABA-A receptor mediate inhibition of the stress response.[46]

Testosterone is the main androgen circulating in humans.[47] It modulates neuronal excitability via genomic and non-genomic mechanisms.[47] Testosterone upregulates expression of neuropeptide Y (NPY), a neuropeptide with anti-stress effects.[48,49] Additionally, metabolites of testosterone (e.g., 3α-androstanediol) may positively affect $GABA_A$ function.[30]

Estradiol, the primary endogenous form of estrogen, is most potent during pre-menopause [50] and is converted from testosterone via aromatase enzymes. Estradiol is produced primarily in the gonads, as well as other organs, including the heart, brain, and skin.[50] Estradiol signals throughout the body via classic nuclear (slow) receptors (ERα and ERβ), as well as more rapid-acting receptors (e.g., GPR30 and ER-X).[50] Estradiol has been shown to have anxiolytic-like effects and to potentiate fear extinction via binding to both ERα and ERβ.[30,51] Furthermore, estradiol modulates the serotonergic system, as well as activity of the HPA axis.[41] Estradiol inhibits expression of the serotonin transporter (SERT), thereby increasing the time serotonin is available to signal in the synapse.[52] Estradiol also modulates levels of adrenocorticotropic hormone (ACTH) and cortisol via actions on glucocorticoid and CRH signaling.[28] Preclinical evidence also shows that estradiol modulates the release of dopamine in the striatum and nucleus accumbens via binding to membrane estradiol receptors, most notably in female rodents.[53,54] Chronic administration of estradiol in female rodents, increases D2 binding in these brain areas via Erβ. However, rapid administration of estradiol heightens amphetamine stimulated striatal-dopamine response in female rodents. It has also been hypothesized that this relationship between estradiol and dopamine may be regulated via GABAergic striatal neurons in females.[54]

DHEA is also derived from pregnenolone via 17α-hydroxypregnenolone.[24,55] DHEA has been suggested to interact with sigma 1 receptors[56] and it is a positive modulator of NDMA receptors.[30,57,58] DHEA also modulates the GABAergic system,[32] with evidence that DHEA and DHEA-S block $GABA_A$ receptors.[30,56] DHEA has been implicated in modulation of HPA axis activity. Ongoing DHEA administration resulted in lower cortisol levels, possibly because DHEA promotes conversion of cortisol to an inactive metabolite.[30] Additionally, administration of DHEA has been associated with increased levels of estradiol, testosterone, and allopregnanolone.[30]

Given the evidence that neurosteroids modulate neurotransmitter systems, such as the GABAergic system, better understanding of the relationship between neurosteroids, PTSD, and AUD has the potential to elucidate novel treatments for these disorders.

3. PTSD

3.1 Pregnenolone

While initial evidence suggested that pregnenolone is an important neurosteroid in the regulation of PTSD symptom severity,[29,30] limited studies have explored this association within the past ten years. Furthermore, a large portion of recent research explores pregnenolone in relation to concurrent PTSD and traumatic brain injury (TBI). For instance, when comparing Veterans with a history of mild TBI (mTBI), both PTSD and mTBI, or healthy civilians, those with both PTSD and mTBI exhibited decreased cortical thickness in the left middle temporal and right orbitofrontal cortex, and this was associated with lower levels of pregnenolone.[59] Decreased

cortical thickness was further associated with heightened PTSD symptom severity.[59] This study included primarily men, no analyses were conducted to evaluate potential sex differences, and it did not include a PTSD only group, which limits conclusions to be drawn about PTSD independent for mTBI. Nonetheless, this demonstrates the potential intersection between pregnenolone levels and PTSD within the context of comorbid mTBI, as this relationship was not observed in the mTBI only or control groups.[59]

It should be noted, however, that the relationship between PTSD and pregnenolone is not consistent among individuals with a history of TBI. Male Veterans who sustained a blast related TBI showed a trend toward lower levels of pregnenolone than Veterans with no TBI, but there were no significant differences in pregnenolone levels between Veterans with PTSD/TBI or TBI only.[60] Furthermore, no significant differences were observed in the association between pregnenolone and white matter microstructure (measured via free-water-corrected fractional anisotropy) nor psychological function between individuals with PTSD/mTBI, mTBI only, and controls.[61] Note that there were no sex-based analyses conducted in this study.[61]

It appears that sex-based analyses may be important among studies of pregnenolone and PTSD. The one study that examined sex differences showed that women with PTSD had higher levels of pregnenolone in postmortem samples of the medial orbital frontal cortex as compared to women who did not have PTSD. Surprisingly, there were no differences in pregnenolone levels in men with or without PTSD.[62]

3.2 Progesterone

There is mounting evidence that progesterone may mitigate the trauma-related symptoms observed in PTSD, including depressive mood and hyperarousal.[23] Recent studies have explored the role of progesterone on trauma-related symptoms, using menstrual cycle subphases as a proxy for measured hormone levels. For instance, during the early follicular phase, when progesterone and estradiol levels are low, women have heightened symptoms, such as depression and “phobic anxiety.” Women with PTSD also experienced more intense anxiety in the early follicular phase, a time when progesterone levels are low, as compared to the midluteal phase, a time when progesterone levels peak, as compared to women without PTSD.[63] However, although women with PTSD endorsed higher levels of anxiety sensitivity compared to trauma-exposed controls, levels of anxiety sensitivity remained stable across the menstrual cycle for both groups of women, not varying based on fluctuations in progesterone.[64] This suggests that generally low levels of progesterone, as proxied via phases of menstrual cycle characterized by low progesterone, are as associated with heightened negative affect. Furthermore, anxiety sensitivity was not impacted by fluctuations in progesterone levels.

When looking at measured hormone levels, there is evidence that higher progesterone levels are related to heightened PTSD vulnerability in clinical and laboratory settings. Plasma progesterone levels, following trauma exposure among men and women presenting to the emergency department, predicted the development of non-remitting PTSD among men, but not women.[65] However, the effect of progesterone on trauma-related symptoms has been observed in both men and women in laboratory-based paradigms. Among healthy women, who were exposed to a violent trauma film, higher salivary levels of progesterone were indicative of more daily intrusive memories the day following the film viewing, but not the subsequent days.[66] Higher saliva levels of progesterone were associated with increased daily intrusive thoughts, following administration of hydrocortisone but not a placebo prior to a distressing film, in healthy men, but not women.[67] This illustrates the potential for an important role of progesterone in regulating stress-reactivity and the likely impact of sex differences between men and women.

The effects of progesterone on stress-response are not consistent in the literature. For instance, following a metyrapone challenge (a laboratory paradigm, which blocks cortisol

synthesis and increases progesterone), increases in progesterone were observed among individuals with PTSD and those without PTSD regardless of sex. Additionally, women, in both the PTSD and control conditions had a greater increase in progesterone as compared to men. The difference in progesterone levels between individuals with and without PTSD was 1.48 times greater among women as compared to men. Interestingly, progesterone mediated the relationship between PTSD and ACTH (a precursor to cortisol) in both men and women, but the direct effect between PTSD and progesterone was positive in women and negative in men.[68] Such sex-specific effects are surprising, as progesterone was previously shown to attenuate cortisol response, in both men and women.[69-71] Similarly when also looking at findings from studies which stratified analyses by sex or only included one sex, progesterone's effect on HPA-axis reactivity in response to a laboratory stressor was observed in women.[71] However, progesterone levels were not associated with PTSD symptom severity in men with PTSD or trauma-exposed controls.[72]

Recent PTSD research has also incorporated concepts from fear conditioning theory, including fear acquisition and extinction into laboratory paradigms to study PTSD symptoms and progesterone levels. Despite this, these studies have not found associations between progesterone (or proxied menstrual cycle phase) and trauma symptoms in such paradigms. Salivary progesterone levels were not related to fear acquisition via conditioned stimulus of a violent film clip or fear extinction in healthy female university students (ages 18-35 years old) in either the follicular or luteal phases of the menstrual cycle, and intrusive memories on the following three evenings were also not associated with progesterone levels.[73] Similarly, in a clinical sample, women with PTSD compared to trauma-exposed controls exhibited less prepulse inhibition (a biomarker of sensorimotor filtering, an underlying mechanism of PTSD, in which a muted stimulus reduces response to a startling stimulus). Despite this, there were no significant differences between the early follicular and mid-luteal phases of the menstrual cycle.[74]

3.3 Allopregnanolone

Emerging evidence suggests that allopregnanolone may be protective against PTSD, as well as other psychiatric disorders[75] such as depression and anxiety.[76] For example, in a sample of military Veterans with PTSD undergoing an evidence-based psychotherapy, higher baseline levels of allopregnanolone were related to the reduction of PTSD symptoms in treatment. Among men with PTSD in the trial, those who responded to the treatment had higher levels of allopregnanolone as compared to those that did not respond to the treatment.[77]

It also appears that the relationship between allopregnanolone and PTSD can be observed in important brain structures in the neurocircuitry of PTSD. In postmortem brain samples of the medial orbital frontal cortex, which has been associated with learning, emotion, and reward behaviors,[78] men with PTSD had lower levels of allopregnanolone compared to controls.[62] Higher allopregnanolone levels were also related to increased whole brain white matter microstructure among individuals with PTSD and mTBI, as compared to individuals with only mTBI.[61] Similarly, Veterans with PTSD and mTBI had decreased cortical thickness, which was associated with reduced allopregnanolone and heightened trauma symptom severity.[59] These studies suggest potential neuroprotective properties of allopregnanolone in the men with PTSD. However, it is important to highlight that these studies did not explore sex differences adequately.

Several recent studies have illustrated a problem in the metabolism of progesterone to allopregnanolone in men with PTSD. Specifically, lower values of the combined allopregnanolone + pregnanolone (ALLO+pregna) assays were related to heightened PTSD symptoms among individuals with PTSD,[72,79] especially dysphoria[79]. Specifically, there was a negative correlation r=-0.59, p=0.01) between the combined ALLO+pregna sum and PTSD

symptoms, as measured by the Clinician-Administered PTSD Scale (CAPS-IV) score,[72] which was replicated in another study ($\rho = -0.74$, $p = 0.006$) utilizing a one-week total (CAPS-IV) score.[79] The later study also observed a PTSD-related decrease in ALLO+pregna to 5α-dihydroprogesterone (5α-DHP) and 5α-DHP to progesterone.[79] In this study ratios of the neurosteroids and its neurosteroid precursor (e.g., ALLO+pregna/5α-DHP) were calculated to indirectly show enzyme activity. The PTSD associated decrease in ALLO+pregna to 5α—DHP is suggestive of a block in 3α-HSD synthesis, whereas the a similar decrease in 5α-DHP/progesterone is potentially indicative of a block of allopregnanolone synthesis via the 5α-reductase.[79] Similarly, the allopregnanolone to 5α-DHP ratio is lower in women with PTSD compared to trauma-exposed controls and women with PTSD also have an attenuated increase in response to a laboratory stressor in both the early follicular and luteal phases.[80] Higher levels of ALLO+pregna are related to improved extinction retention (utilizing an average of conditioned stimuli trials on the second testing day) to a laboratory stressor (a mild electric pulse) in women with PTSD, but this was not observed in trauma-exposed women without PTSD.[81] Comparable to studies among men,[79] the ALLO+pregna to 5α-DHP ratio suggested that conversion to allopregnanolone was blocked in women with PTSD.[81] These findings suggests that metabolism of progesterone to allopregnanolone may warrant additional study, especially among populations including both men and women.

The conversion of progesterone to allopregnanolone also affected the ACTH pathway. Following a metyrapone challenge in the laboratory, to increase ACTH levels in men and women with PTSD as compared to controls, increases in allopregnanolone were observed across groups. Specifically, women showed a 1.33-fold greater increase in allopregnanolone response versus men and allopregnanolone regardless of PTSD diagnosis. There were no differences between individuals with PTSD versus controls. Levels of allopregnanolone both directly and indirectly mediated the relationship between PTSD and ACTH in both men and women, .[68] Thus, ALLO may be an integral component in the stress-response system in both men and women with PTSD.

3.4 Pregnanolone

Similar to the other progesterone metabolites, high levels of pregnanolone have been associated with improved psychological functioning and reduced distress.[23] Despite this, few recent studies have included analysis of pregnanolone levels. During a trial of web-based prolonged exposure therapy (PE) there was no association between pregnanolone and PTSD severity at baseline. However, higher pregnanolone levels were associated with a trend toward greater slope in PTSD severity. Interestingly, when men were separated in the analysis by sex, men who responded to the web-based treatment had higher levels of pregnanolone than non-responders.[77]

Increasingly, research is exploring pregnanolone in relation to other neurosteroids (i.e., ALLO+pregna). Among men with PTSD, levels of ALLO+pregna in cerebral spinal fluid were negatively associated with PTSD symptoms, in that increased levels of ALLO+pregna were correlated with less PTSD symptoms, as measured by the CAPS-5.[79] This relationship remained when considering the ratio of ALLO+pregna to DHEA ratio.[79] As mentioned in the above section, ALLO+pregna levels accounted for 47% of the variance in PTSD symptom scores in men.[72] Notably, these studies did not evaluate ALLO+pregna in women.

3.5 Testosterone

Recent research has begun to explore the relationship between PTSD symptoms and testosterone, following combat zone deployment.[82] Plasma testosterone increased in men after deployment as compared to baseline levels and lower pre-deployment levels of testosterone were related to increased PTSD symptoms 1- and 2-years following deployment.[83] Conversely, several other recent studies have shown that testosterone levels do not differ between men with and without PTSD. When examining a group of combat Veterans diagnosed with PTSD,

Veterans without PTSD, and a group of non-combat exposed civilians, male Veterans without a PTSD diagnosis had higher levels of testosterone compared to the control group. There were no differences in testosterone levels among male Veterans with PTSD and those without PTSD;[84,85] nor did testosterone levels predict vulnerability to non-remitting PTSD in men or women.[65] Additionally, both total or free testosterone levels in male Special Forces Operators in the United States (US) military remained stable pre- and post a three to six month deployment.[86] These studies illustrate the heterogenous findings among testosterone levels and PTSD, with several studies failing to demonstrate changes in testosterone in relation to PTSD.

However, despite these negative results, laboratory studies highlight a potential relationship between testosterone and PTSD. When examining a predominantly male sample of US Veterans, who completed a pre-deployment stress reactivity challenge, soldiers who demonstrated a blunted cortisol and testosterone response to the laboratory challenge endorsed heightened PTSD symptoms after deployment. There was also a linear relationship between the average of monthly traumas experienced while deployed, cortisol, and PTSD symptoms.[87] In this same group of soldiers, those with low testosterone levels had increased risk of depression when considering potentially traumatic exposures from deployment and higher increases in levels of testosterone reactivity were indicative of a higher risk of depressive symptoms.[88] The one study that included young women with PTSD demonstrated that, following a laboratory stress induction, testosterone levels were elevated in women with PTSD. Increased levels were also observed in both healthy controls and women with borderline personality disorder.[89] Accordingly, in women and men low testosterone is linked to more depressive symptoms and higher testosterone levels are also tied to similar results, indicating that optimal homeostatic levels of testosterone are an important component of mood regulation.

Additionally, there is evidence that the effects of testosterone may extend trauma-related symptoms. In a small sample of male soldiers, there were no differences in testosterone levels when soldiers with PTSD were compared to soldiers without PTSD. However, when analyses considered other concurrent psychiatric co-morbidities, soldiers with only PTSD had higher levels of testosterone than those with co-morbid major depressive disorder or alcohol use, as well as the control group. The comorbid condition groups and the control group did not significantly differ in testosterone levels.[90] Such discordant findings across various mental health diagnoses demonstrate that while testosterone may be related to psychiatric symptoms, the relationship between testosterone levels and these diagnoses and symptoms is unclear and warrants continued research.

3.6 Estradiol

Recent evidence provides an unclear relationship between estradiol levels and PTSD symptoms. Lower estradiol levels were associated with increased reexperiencing symptoms and avoidance symptom severity in naturally-cycling, trauma-exposed women. Additionally, when exposed to a trauma reminder, women with lower estradiol levels had higher subjective reactivity and lower cortisol reactivity compared to women with higher estradiol levels.[91] Lower estradiol levels have also been associated with increased intrusive memories following exposure to an experimental trauma film in healthy women,[66] as well as heightened skin conductance responses during fear extinction.[73,93] However, estradiol levels predicted using a machine learning DNA methylation predictor, which were highly correlated with actual measured estradiol samples within a testing set, were negatively correlated with current PTSD and trauma symptom severity in women, meaning higher predicted estradiol levels were associated with increased PTSD symptoms.[92] In contrast, high levels of estradiol are protective against severe trauma-related symptoms associated with fear habituation.[93]

When using the menstrual cycle as a marker for estradiol levels there are similar discordant findings. PTSD symptom severity was also lower in women with lower estradiol levels and a history of sexual trauma, as compared to nonsexual-based traumas, and trauma-related symptoms fluctuated across the menstrual cycle, with higher symptoms during times of low estradiol.[94] Women report heightened symptom severity, including increased depressive symptoms and "phobic anxiety," during the early follicular phase (days 2-6, defined by lower estradiol levels) as compared to the midluteal phase (6-10 days after ovulation, defined by high levels of estradiol and progesterone) and when separated by PTSD status, women with PTSD experienced heightened "phobic anxiety" in the early follicular phase.[63] Low estradiol has also been associated with heightened fear reactivity during laboratory-induced fear extinction in women with PTSD as compared to controls,[95] and impaired fear inhibition was similarly observed among healthy women in the follicular phase.[96]

Conversely, additional studies have demonstrated that the midluteal phase is also associated with PTSD symptomatology.[97] In one study, daily PTSD symptoms fluctuated across the menstrual cycle, with symptoms increasing as individuals moved from a low- to a high-estradiol phase when tracking symptoms using ecological momentary assessments.[91] Furthermore, women with PTSD in the mid-luteal phase had poorer retention of extinction learning (as measured via an average of conditioned stimuli trials on the second day of testing) compared to women in the early follicular phase. Conversely for women in the control condition, the midluteal phase was associated with improved extinction retention.[98] In a separate study, women in the early luteal phase, experienced the most intrusions after viewing a traumatic film.[99] Similarly, high levels of estradiol were associated with an increased likelihood of intrusive thoughts at the beginning of the week after women watched a distressing film, but then decreased at the end of the week.[100] Higher estradiol levels were also positively associated with activity in the rostral anterior cingulate (generally associated with fear processing[101]) and ventromedial prefrontal cortexes (generally associated with PTSD-related emotional processing[102]) during viewing of trauma-related videos among women in both the follicular and luteal phases, who were not on hormonal contraceptives (mean estradiol= 4.36pg/mL, SD=1.36).[103] Similarly, the preclinical literature suggests that estradiol modulates extinction of fear memory in rodents, and there is some evidence that estradiol may attenuate fear acquisition (for review see Maeng & Milad, 2015[104]).

Clinical research has also explored the impact of hormonal contraceptives containing estrogens and progestins on trauma-related symptoms. Women receiving emergency contraceptives and women already taking hormonal contraceptives, experienced fewer intrusive symptoms following sexual assault. Specifically, women taking Ogetrel (an emergency contraceptive, combination of estrogen ethinyl estradiol and progestin levonorgestrel, administered in two oral doses) experienced less trauma-related symptoms than other emergency contraceptives or those who declined.[105] Among women taking hormonal contraceptives, there was improved acquisition and extinction of fear as compared to women not taking contraceptives and men.[106] However, women using hormonal contraceptives exhibited poor extinction recall versus naturally-cycling women,[107] as well as higher acquisition of fear conditioning and extinction versus women not on hormonal contraceptives or men.[106] Additionally, estradiol levels in free-cycling women were positively associated with ventromedial prefrontal activity while viewing a traumatic film, whereas women taking hormonal contraceptives had heightened activity in the insula and dorsal anterior cingulate as compared to women not taking hormonal contraceptives.[103]

The relationship between estradiol and PTSD among men is complex. Estradiol did not differ significantly between male Veterans with or without PTSD.[85] However, following exposure

to a traumatic event, men with higher concentrations of estradiol had increased concentrations of circulating pro-inflammatory cytokines, which in turn were related to lower probability of non-remitting PTSD.[65]

The relationship between low estradiol and trauma-related symptoms has been observed in both men and women. For instance, stress-exposed and healthy individuals completed a psychosocial stressor versus a control task in the laboratory to explore the effects of estradiol on fear conditioning. During delayed fear extinction task, women in the early follicular phase (defined by low estradiol levels) had more fear responses in the stress-exposed condition than controls.; In the stress-exposed condition women who were identified as being "midcycle" (high levels of estradiol and progesterone) had fewer fear responses than controls, suggesting impaired fear extinction in women when estradiol is low.[108] Interestingly, intrusive thoughts related to exposure to a traumatic film were lower in men with higher baseline estradiol compared to women with high estradiol.[67]

3.7 DHEA/DHEA-S

DHEA and DHEA-S appear to be altered across various clinical populations with PTSD.[109] For instance, male combat Veterans with PTSD had higher levels of DHEA-S as compared to age-matched controls without PTSD[110] and postmenopausal women with significant physical/sexual abuse histories had increased levels of DHEA throughout the day.[111] Similarly, among adult trauma survivors, higher acute DHEA-S level, as well as a lower cortisol to DHEAS-S ratio, predicted trauma-related symptoms six-weeks following the trauma, but this relationship was not sustained at the six month follow-up.[112]

While these recent studies have identified higher levels of DHEA/DHEA-S among individuals with PTSD, there are also studies that illustrate the opposite relationship. In one study, there was no differences in DHEA or DHEA-S in cerebral spinal fluid between women with or without PTSD.[79] Female adolescents with PTSD who were exposed to a sexual-related trauma, were found to have lower DHEA-S compared to controls, and this was associated with depression.[113] Among mostly males with military service, blood DHEA levels were decreased following captive survival training and history of trauma exposure was not related to DHEA levels.[114] Similarly, when including both sexes, adults with a history of childhood trauma exhibited a lower cortisol/DHEA ratio, but the cortisol/DHEA ratio was independent of PTSD diagnosis.[115]

Interestingly, the relationship between cortisol and DHEA/DHEA-S has been shown to be important for stress-reactivity. In a group of female and male smokers both with and without PTSD, DHEA-S levels were inversely related with anxiety sensitivity and negative affect.[116] Additionally, among female survivors of intimate partner violence (IPV), women who had depressive symptoms had higher DHEA levels in both the morning and evening. However, the ratio of cortisol to DHEA was lower in this group in the morning as compared to all other groups, including women survivors of IPV with PTSD.[117] There have been other studies that have found no differences in DHEA/DHEA-S response following administration of agents to stimulate HPA-reactivity. At baseline female adolescent rape survivors had lower DHEA-S levels as compared to controls, but there were no group differences following administration of dexamethasone (a synthetic glucocorticoid receptor agonist, which is used in laboratory studies to probe negative feedback of the HPA axis loop).[118] Additionally, there were no significant differences in DHEA response between male Veterans with PTSD, Veterans with trauma-exposure but no PTSD, and healthy Veteran controls following an injection of cosyntropin (synthetic ACTH).[119]

There is also a relationship between DHEA and the progesterone-derived metabolites, ALLO+pregna. In a study of women with and without PTSD who completed a fear conditioning paradigm, there was a trend level relationship between a low ratio of ALLO+pregna to DHEA and extinction retention during the early follicular phase, a time when DHEA levels are similar to

allopregnanolone and pregnanolone.[81] However, among women with PTSD, lower ratios of both ALLO+pregna to DHEA and ALLO+pregna to DHEA-S were related to heightened PTSD symptoms, including dysphoric trauma-related symptoms.[79]

Finally, DHEA/DHEA-S were not associated with PTSD severity nor changes in PTSD symptoms following completion of a treatment course of an evidence-based psychotherapy, including web-based prolonged exposure,[77] Eye Movement Desensitization Retraining (EMDR),[120,121] or a mindfulness intervention.[122] This suggests that DHEA and DHEA-S levels likely do not change following treatment.

3.8 PTSD Summary

Taken together, recent literature illustrates the complex relationship between neurosteroids and PTSD. This relationship is plagued by discordant findings and likely reflects differences between men and women with PTSD, as well as various populations (e.g., Veterans/civilians) and methodologies to study PTSD and trauma-related variables. Additionally, many of the studies use subphases of the menstrual cycle, which can be challenging to interpret across studies.[97] Most importantly, most studies did not employ SABV in study design or analyses (See Table 1). Future research among women, also needs to account for hormonal fluctuations across reproductive stages, including pre-, peri-, and postmenopause. Despite these difficulties, there is emerging evidence that pregnenolone-derived neurosteroids, specifically progesterone and allopregnanolone, mitigate PTSD symptoms, diagnoses, and treatment response (See Table 2). Thus, these neurosteroids warrant additional research regarding their utility to treat PTSD. Conversely, results are mixed among estradiol, testosterone, and DHEA/DHEA-S. Overall, further research is needed to explore the relationship between these neurosteroids and PTSD. .

4. Alcohol

4.1 Pregnenolone

Few recent studies have explored the relationship between pregnenolone and alcohol consumption, which higher pregnenolone levels associated with alcohol-related variables. For instance, following consumption of three standard alcoholic drinks in a human laboratory study, levels of pregnenolone increased by 26% across the whole sample. When looking at sex differences, men showed a trend-level ($p<0.10$) increase in pregnenolone levels after drinking the alcohol.[123] Interestingly, among the sex stratified analyses, pregnenolone concentration was positively correlated with subjective “liking” of alcohol in men, but no significant associations were observed among women.[123]

When looking at postmortem brain tissue of men and women with an AUD, higher pregnenolone levels were observed in all brain regions except the amygdala. In fact, pregnenolone levels were 71-164% higher among individuals with AUD as compared to healthy controls. Women with a history of AUD had similar pregnenolone levels as health controls. However, there were only two women included in the study who had a history of AUD.[124]

4.2 Progesterone

Endogenous progesterone has been related to alcohol consumption and use across a range of clinical studies.[23] High levels of progesterone may be protective against abuse-related effects of alcohol and other substances[41] and recent data further supports the possibility that progesterone may mitigate alcohol consumption and administration [23]. For instance, recent studies of women college students have demonstrated that higher progesterone levels are associated with less alcohol consumption.[125,126] Over the course of two weeks, increases in salivary progesterone levels in young women were positively associated with decreases in the

likelihood of drinking. In fact, for each standard deviation increase in progesterone, there was a 1.61 decrease in the likelihood of alcohol use. Additionally, mood states moderated this relationship. Women who rated their mood as negative during a day on which there were low levels of progesterone were more likely to drink as compared to days when mood was rated positively.[126] Furthermore, in naturally cycling college-aged women, binge drinking risk was low and overall drinking risk was moderate during the midluteal phase,[125] a time when progesterone is at its peak in the menstrual cycle.[97]

When tracking daily alcohol use and motives for drinking among adult women, there is a decrease in drinking during the mid-cycle, when progesterone levels are rising. There was also a trend toward increasing drinking for social motives in the late follicular/ovulatory phases (days 5-16), when estradiol has peaked[97]. Notably, drinking to cope with negative affect has been related to the amount of alcohol consumed during days 1-5 of the menstrual cycle[127], when both progesterone and estradiol levels are low.[97] Relationships between progesterone levels and alcohol use are also observed across reproductive stages. Among postmenopausal women, higher levels of progesterone were related to lower alcohol craving and craving frequency during alcohol withdrawal. Additionally, postmenopausal women reported higher overall craving compared to their premenopausal counterparts.[128] Interestingly, men with AUD had higher levels of progesterone compared to men without AUD in this study.[128]

Despite these results illustrating that progesterone may mitigate alcohol use, additional research shows that this relationship is not clear. For example, regularly cycling women in treatment for AUD consumed more alcohol during the mid-late luteal phase, a time when progesterone is high and estradiol is increasing,[97] as well as during menses, a time when both estradiol and progesterone are at their nadir.[97,129] However, among those identified as having lower baseline depression, individuals who reported high distress during the mid-late luteal and/or menses had increased craving intensity during menses, whereas women reporting lower distress during those phases endorsed heightened craving intensity during the mid-late luteal and menses phases.[129]

Similarly, women were more likely to drink during the midluteal phase (days -9 to -5 prior to menses, typically defined by higher progesterone levels) than during the perimenstrual phase (days −3 to +2 in relation to menses onset, typically defined by low progesterone and low estradiol). In the same study, women in the periovulatory (days −2 to +1 in relation to LH-surge, when there are rising progesterone and estradiol) and perimenstrual phases were more likely to drink heavily on weekdays as compared to the midluteal phase. Drinking to cope was higher in the perimenstrual phase whereas drinking for social reasons was high for weekend drinking across almost all phases, except perimenstrual.[130]

4.3 Allopregnanolone

There has been increasing interest in exploring the possibility of allopregnanolone as a novel treatment target for AUD.[29,131,132] However, few studies within the past ten years have explored the effects of allopregnanolone on alcohol use in clinical populations. Studies of postmortem brain tissue from individuals with AUD have demonstrated higher levels of allopregnanolone in the ventral tegmental area (a dopaminergic brain area implicated in alcohol reward) when compared to brain tissue from social drinkers, who consumed on average 1-2 drinks per day. Males with AUD also had higher levels of allopregnanolone in the substantia nigra pars medialis (SNM; another dopaminergic brain area implicated in reward), but not among women with AUD. Brain tissue from female social drinkers had higher levels of allopregnanolone in the SNM compared to male social drinkers.[133] Conversely, results from a different study determined that men with AUD had an approximately 60% lower allopregnanolone levels in postmortem cerebellum tissue compared to controls.[134] Across the sample (including men with and without AUD), daily and weekly alcohol consumption was negatively associated with allopregnanolone levels in the cerebellum.[134]

Additionally, the relationship between levels of allopregnanolone and alcohol consumption have also been illustrated within laboratory settings. However, such studies have produced heterogenous results. For instance, women with severe premenstrual dysphoric disorder (PMDD) and healthy female controls who were administered a 30-minute alcohol infusion (0.2g/kg) showed lower levels of allopregnanolone, specifically in the late luteal phase.[135] Low levels of allopregnanolone following consumption of alcohol were also observed among men and women with no substance use history. In fact, 40 minutes after participants drank 3 standard grain-alcohol drinks, allopregnanolone levels were lowered by approximately 30% across sexes and allopregnanolone levels were associated with higher liking and wanting of alcohol in men.[123] Allopregnanolone levels also decreased by approximately 20% following a low-dose alcohol infusion (0.06g/dl) in a laboratory study of thirty-two non-treatment seeking heavy drinkers administered naltrexone (50 mg/day for three days) or placebo, regardless of medicationadministration.[136] Another study of healthy men and women showed that 0.8g/kg (for men) or 0.7g/kg (for women) of alcohol did not change allopregnanolone levels in women in the luteal phase and similarly did not impact levels during the follicular phase or in men.[137]Additionally, a recent study demonstrating that naltrexone increased allopregnanolone by almost 50% among individuals with the Asp40 allele of the OPRM1 gene, which is thought to influence phenotypes related to AUD.[136] Thus, while there is a relationship between allopregnanolone levels and alcohol use in laboratory settings, the directionality of this relationship is unclear in the available literature to date.

4.4 Pregnanolone

Few recent studies have included analysis of pregnanolone in relation to alcohol consumption, symptomatology, or treatment. In fact, we only identified one study exploring the relationship between pregnanolone and alcohol in the time period covered by this review. In this study of postmortem brain tissue, men with AUD had approximately 60% lower pregnanolone levels in the cerebellum compared to controls.[134] The limited inclusion of pregnanolone in the current literature is consistent with the sparse historical exploration of pregnanolone in studies of alcohol consumption.[23]

4.5 Estradiol

Historically, evidence regarding an association between estradiol and alcohol consumption has been mixed,[23] with some studies showing increases in estradiol and estrogen metabolites following alcohol intake[138,139] whereas other studies have shown no changes.[137] In general, recent research suggests alcohol is related to heightened estradiol levels. For instance, among women who consumed more than 20 grams of alcohol per day, levels of estradiol and estrone (another type of estrogen that is associated with postmenopause) during the luteal phase were higher than nondrinkers. Among women consuming more than 10 grams of alcohol per day, follicular estrone sulfate was higher than non-drinkers.[140] Total alcohol consumption has also been associated with higher urinary estradiol levels. Indeed, estradiol levels were 22% higher among women consuming 5 or more glasses of wine per week as compared to women who did not drink.[141] The association between estradiol and alcohol during premenopause is linear. Total and free estradiol increases 5.26-5.82% for every drink consumed.[142] Additionally higher estradiol and lower progesterone levels are related to increased alcohol consumption and binge drinking.[125] Higher levels of estradiol are positively related to increased alcohol use in the early and mid-luteal phase, increasing by 1.9 to 2.4% respectively. A similar relationship is also observed among postmenopausal women taking hormone replacement therapies. Estradiol and other estrogen-based metabolites increased with

increasing alcohol intake[143] and liquor consumption was related to higher estradiol levels in postmenopausal women not taking hormonal replacement therapies.[143]

When looking at the menstrual cycle, naturally-cycling women endorse higher alcohol craving during the follicular phase, when estradiol may be expected to be elevated as compared to the luteal phase, when estradiol is elevated along with progesterone.[144] Women on hormonal contraceptives, which generally stabilize fluctuating hormone levels, consumed more alcohol compared to naturally-cycling women.[144] Healthy women who were administered 0.6g/kg of alcohol during two sessions (early versus late follicular phase, when estradiol levels are low and then peak respectively), reported that alcohol-induced disinhibition (as measured via a cued go/no-go reaction time task) was doubled during the late follicular phase.[145] Women have also shown that 'drinking to cope' predicted alcohol consumption during the menstrual phase (days 1-5), when both estradiol and progesterone are low, and 'social motives to drink' peaked during days 5-16 of the cycle, when estradiol is rising.[127] These studies demonstrate that women may drink for different reasons as levels of estradiol fluctuate across the menstrual cycle.

Recent research also has highlighted potential sexually dimorphic relationships between alcohol exposure and estradiol levels. Increases in estradiol have been observed among women following an acute, intravenous alcohol infusion whereas estradiol levels decreased in males as compared to a placebo infusion.[146] Specifically, young females (ages 21-25) had a 36-fold increase in estradiol following alcohol administration versus placebo, whereas older females (ages 55-65) had a 3.2-fold increase. Both older and younger males had decreases in estradiol levels following alcohol administration as compared to placebo (4-fold and 2.3-fold, respectively).[146] In men, estradiol levels were not related to alcohol intake (binge drinking or recent intake) the week before.[147] Among adolescents, higher saliva estradiol levels were associated with increased alcohol consumption and early onset of use among boys.This o relationship, between alcohol use and estradiol levels, was not observed in adolescent girls.[148,149]

4.6 Testosterone

The relationship between testosterone and alcohol use has been illustrated in the literature to date, with evidence showing that increased testosterone levels are associated with heightened alcohol consumption in men (for review see Erol et al., 2019).[150] Recent evidence supports this association. For example, alcohol-related changes in testosterone were observed in a large study (n=1,221) of Danish men entering military service. Higher levels of free testosterone were associated with increased levels of alcohol consumption in the preceding week.[147] Additionally, when comparing groups of males experiencing alcohol-related withdrawal and healthy controls, those with AUD had significantly higher serum testosterone levels. Serum testosterone levels decreased over the course of detoxification and were positively correlated with alcohol craving.[151] Conversely, following an infusion of either alcohol or saline (placebo), alcohol decreased testosterone levels two-fold relative to placebo infusions among both young (21-25 years old) and older (55-65 year old men). , There was a small increase in testosterone levels in young women and a 2 fold increase among older women versus the placebo condition.[146] It should be noted that postmortem studies of brains from men and women with AUD have not showed differences in levels of testosterone as compared to controls.[124]

The association between testosterone and alcohol use is also observed in adolescent boys and young adults. Higher levels of testosterone were associated with an increase in likelihood of lifetime alcohol use among boys (ages 12-17 years old).However, testosterone levels were not related to alcohol measures, such as quantity of alcohol consumed across their lifetime or recent alcohol use.[148] This suggests an earlier onset of alcohol use in adolescent

boys with higher testosterone levels. In the same study, there was no relationship between testosterone levels and alcohol use among girls.[148] Boys (ages 12-25 years old) had increased recent and lifetime alcohol use linked to reduced amygdala-orbitofrontal cortex (OFC) connectivity,[152] which is related to poor emotion regulation.[153] Higher testosterone levels were associated with reduced functional connectivity between the amygdala and the OFC, which was associated with increased alcohol use. It should be noted that there was no mediation effect of testosterone, amygdala-OFC connectivity, and alcohol use in girls.[152] When grouping both boys and girls together (controlling for menstrual fluctuations), higher levels of salivary testosterone were predictive of increased alcohol use two years later.[154]

Despite most of the evidence to date suggesting a relationship between higher testosterone levels and alcohol use among men, the relationship between testosterone and alcohol is somewhat complex in women. Free testosterone levels increased by 1.42% for each alcoholic drink consumed in healthy premenopausal women tracking alcohol use via dietary recall.[142] Furthermore, women consuming at least 20 grams of alcohol per day had 23% higher testosterone levels compared to women with no alcohol use.[155] This relationship between testosterone levels and alcohol use is linear and is observed in both pre- and postmenopausal women. Testosterone levels increased by 3.9% in premenopausal women and 2.3% in postmenopausal women in response to each increment of 10 grams of alcohol per day.[149] Conversely, in another study free testosterone levels were approximately 18% lower for women consuming more than 20 grams of alcohol per day compared to non-drinkers, and increased alcohol use was overall associated with decreased free testosterone levels.[140] Additionally, premenopausal women with self-reported moderate alcohol use (15-20 grams of alcohol per day, 2-5 drinking occasions per week for the past 3-5 years) did not show increased testosterone levels.[156] In older women across the menopausal transition identified as excessive drinkers, higher levels of testosterone were associated with a lower likelihood of transitioning to non-excessive drinking.[157] This relationship was not observed among non-excessive drinkers transitioning to problematic drinking,[157] suggesting that that chronic, high-level alcohol use may be related to higher levels of testosterone.

4.7 DHEA/DHEA-S

Similar to testosterone, levels of DHEA and DHEA-S have been shown to be related to increased alcohol use in men.[23] DHEA-S levels increase in parallel with alcohol consumption among men[158,159] and this association is observed even in those with moderate alcohol consumption.[159] Higher DHEA-S levels in healthy, middle-aged males are positively correlated with alcohol consumption, even when controlling for age. The relationship between problematic alcohol use, as measured by the Alcohol Use Disorders Identification Test (AUDIT), and DHEA-S was observed in men identified as having increased impulsivity, but not those scoring low on impulsivity measures.[159] Furthermore, when looking at postmortem brain tissues of men and women with AUD, DHEA levels are 71-161% higher versus individuals without AUD.[124]

Elevated DHEA levels are also observed among women following alcohol drinking. DHEA levels are 14-23% higher in premenopausal women who consume at least 20 grams per day versus women who do not drink.[155] Additionally, the type of alcohol consumed may impact DHEA levels. For instance, women who consume at least 5 glasses of beer per week have DHEA levels that are 5.1% higher than non-drinkers, but elevated DHEA levels were not observed in response to overall alcohol consumption or wine consumption.[140]

DHEA levels are associated with stress-response in the laboratory, which is a risk factor for AUD. Following exposure to a laboratory stressor, salivary DHEA levels were higher in college-aged women reporting drinking to cope with stress. However,owever physiological stress response (e.g. cardiovascular measures) was dampened in those with higher DHEA levels.[160] When healthy young men (ages 21-40 years old) were exposed to a laboratory stressor and then consumed two cans of beer, the rise and fall of DHEA levels were not

significant and the levels remained stable after consumption of alcohol.[161] Conversely, DHEA-S levels were 70% lower among women with AUD, who were hospitalized, versus controls whereas there were no baseline differences between men with AUD, who were hospitalized, and controls. A decline in DHEA-S levels was observed during early abstinence in both men and women, but DHEA-S levels were only related to lower cravings in women.[162]

Furthermore, DHEA-S results may be race dependent, as research shows that among individuals with likely AUD, African Americans had lower levels of DHEA-S as compared to White counterparts.[163] In another study, the interaction between increased prior day cigarettes and alcohol use was associated with DHEA levels in rural African Americans.[164] Similarly, DHEA-S increased in relation to increased alcohol consumption and tobacco smoking among Japanese men.[158]

4.8 Alcohol Summary

In parallel to the recent literature in PTSD, and allopregnanolone appears to reduce alcohol consumption, and this is likely to be mediated through mitigation of negative affect and stress. Results remain mixed for progesterone, whereas scant research demonstrates that pregnenolone is related to increased alcohol use and cravings (see Table 2). Additionally, levels of estradiol, testosterone and DHEA appear to be related to increased alcohol consumption. Nonetheless, it is important to note that these findings potentially differ between men and women. This body of research is similarly marred by the inconsistent evaluation of SABV, which make it difficult to generalize results across sexes and reproductive stages (see Table 3). Additional research is needed to elucidate the complex relationship between neurosteroids and alcohol use, considering menstrual cycle phases, reproductive stages (pre-, peri- and postmenopause), smoking status, as well as other racial and age variables.

5. Neurosteroids as novel treatments: Pregnenolone, progesterone, and allopregnanolone

Given the association between PTSD symptoms, alcohol consumption, and neurosteroids, specifically with evidence that allopregnanolone may mitigate trauma-related symptoms while reducing alcohol consumption, it is unsurprising that recent studies have begun to study the utility of administering exogenous neurosteroids, including allopregnanolone and its precursors pregnenolone and progesterone, as novel therapeutics for PTSD and AUD. The following sections will review the current evidence for exogenous pregnenolone, progesterone, and allopregnanolone as novel treatments for concurrent PTSD and AUD.

5.1 Pregnenolone

Emerging evidence suggests that administration of exogenous pregnenolone may mitigate stress-reactivity and alcohol craving. For instance, adults (n=31 men; n=12 women) seeking treatment for AUD were randomized to receive exogenous pregnenolone (300 or 500mg per day) or placebo medication for eight weeks. During the second week of the medication trial, participants were exposed to a stress, neutral, and alcohol mood induction paradigm across three days. Those administered pregnenolone had an attenuated increase in stress and alcohol-cue craving as compared to the placebo condition.[165] Additionally, administration of exogenous pregnenolone reduced stress-induced anxiety as compared to individuals administered placebo medication. Pregnenolone normalized cortisol and ACTH levels, in that both doses of pregnenolone elevated cortisol and ACTH in individuals with AUD.[165] These results have been replicated among adult men and women (n=30, n=21 men; n=9 women) with cocaine use disorder, as exogenous pregnenolone reduced craving as well as

stress-induced anxiety.[166] These data demonstrate that pregnenolone may be a worthwhile treatment for improving HPA axis regulation and reducing alcohol craving.

Pregnenolone has also shown promise for attenuating PTSD symptoms. Thirty US male Veterans with a mTBI, who had been deployed to Iraq or Afghanistan, were administered pregnenolone for 8 weeks (titrated to 250mg twice per day) showed significant improvement in trauma-related symptoms, including sleep disturbances, concentration problems, irritability, and hyperarousal.[60] Oral pregnenolone has also reduced depressive symptoms in adults (n=80) aged 18 to 75 years old with bipolar disorder[167] and enhanced connectivity between the amygdala and dorsal medial prefrontal cortex, which was related to decreased anxiety, in male participants aged 18 to 32 years old (n=31).[168] This evidence further highlights that exogenous pregnenolone may mitigate negative affect and improve stress-reactivity. Despite these promising findings, no studies have explored the utility of exogenous pregnenolone to target concurrent PTSD and AUD.

5.2. Progesterone

Among the neurosteroids, administration of exogenous progesterone has been perhaps the most widely studied in substance use disorders (for review see Peltier & Sofuoglu, 2019[41]). While much of this research has occurred outside the ten-year span of the current review, it is important to highlight that exogenous progesterone can reduce drug effects, use, craving and urges across substances.

Progesterone administration is associated with attenuated cravings and reduced positive subjective ratings of nicotine administration, among men and women[169-171] as well as following cocaine administration.[172-174] Clinical trials of exogenous progesterone administration illustrate its potential to increase abstinence, especially among postpartum women who use cocaine or nicotine. For instance, administration of 100 mg progesterone per day for 12 weeks reduced probability of urine drug screens positive for cocaine in postpartum women.[175] Similarly, administration of 400 mg progesterone increased abstinence rates among postpartum women who smoke.[176,177] Notably, chronic administration of 400 mg of progesterone over seven days was related to improved emotional processing in men and women in early abstinence from cocaine use disorder who also misused alcohol.[178]

Progesterone administration has also shown promise in attenuating stress-reactivity. [41] Intramuscular injections of 50 mg progesterone reduced stress-reactivity in response to a laboratory stress paradigm (i.e., Trier Social Stress Test), in healthy men. Specifically, 50 mg and 100 mg doses of progesterone reduced peak cortisol levels, as well as subjective ratings of negative mood and alertness following the Trier Social Stress Test.Progesterone administration did increase blood pressure and plasma noradrenaline simultaneously.[70] In a trial of men and women with cocaine use disorder, those receiving 400 mg of progesterone per day for seven days showed decreased negative emotion ratings and cortisol levels in response to a cue-induction, as well as increased cue-induced ACTH response as compared to individuals receiving placebo treatment.[174] In fact, women receiving placebo medications reported lower ratings of positive mood and negative emotion after the cue-induction.[174] Healthy women pre-treated with 200 mg of oral progesterone endorsed decreased state anxiety following administration of oral d-amphetamine.[179] Medroxyprogesterone, a synthetic progestin, reduced panic/anxiety symptoms in women with panic disorder.[180] Furthermore, members of our group have recently shown that administering progesterone (200mg, twice per day) reduces stress-induced alcohol craving, anxiety and negative affect (i.e., fear, sadness; Ralevski et al., unpublished results) among men with PTSD and AUD[181]. Thus, progesterone may attenuate

stress-reactivity and negative affect, which underlies both PTSD and AUD in both men and women.

5.3 Allopregnanolone

Allopregnanolone is a promising pharmacological target.It has demonstrated significant anxiolytic and antidepressant effects related to stress-reactivity, trauma symptoms and alcohol use. Furthermore, allopregnanolone is well tolerated among women with PTSD, as women with PTSD reported less sedation than healthy controls.[182] This is notable as administration of benzodiazepines, such as diazepam, result in increased sedation in women with or without PTSD, but women receiving exogenous allopregnanolone reported no significant side effects.[182]

An exogenous, synthetic form of allopregnanolone, brexanolone, was approved by the US Food and Drug Administration (FDA) in 2019 for the treatment of postpartum depression (PPD). In Phase III clinical trials, women diagnosed with PPD showed a large, clinically significant reduction in depressive symptoms following a single 60-hour infusion of brexanolone compared to those women receiving placebo injections and these improvements were carried through thirty-day follow-ups. In addition to these positive results, brexanolone was shown to be well tolerated.[183] These study results were also replicated in two additional Phase III trials.[184] Additionally, zuranolone, an oral synthetic form of allopregnanolone, has been shown to reduce depressive symptoms in women with PPD and its effects lasted through the 45 day follow-up.[185] In the initial Phase III study of zuranolone, individuals with major depression, receiving 30 mg of the drug, who also had measurable blood concentrations of zuranolone and severe baseline depressive symptoms, showed significant improvement. This illustrates the potential of zuranolone to treat mood symptoms among patients with significant major depression .[186]

Despite this promising evidence, it is worth noting that a recent study of ganaxolone, a synthetic derivative of allopregnanolone, failed to show improvement in PTSD symptoms. Veterans and civilians received either placebo or ganaxolone (titrated to 60 mg twice per day for two weeks). Although, ganaxolone proved to be both safe and feasible to administer to participants, it did not reduce trauma symptoms.[187] However, this study included mostly males and low trough blood levels of the study drug were found in more than one-third of participants, indicating the need for higher dosing.[187,188]

While there are no clinical studies exploring the administration of allopregnanolone on alcohol consumption and alcohol-related variables (e.g., craving, treatment response), there is promising evidence that allopregnanolone may be an advantageous treatment for drinking. There is significant preclinical evidence that allopregnanolone decreases acquisition, intake, and reinforcement of ethanol.[131] Allopregnanolone reduced consumption of ethanol among rats that preferred ethanol,[131,189] including when administered a precursor to allopregnanolone (e.g., pregnenolone),[190] which has similarly demonstrated promise in humans.[165]

6. Summary and future directions

This review covers the growing literature detailing the effects of neurosteroids on PTSD and AUD symptoms, and the potential for neurosteroids to mitigate the negative affect and stress-reactivity observed in both disorders. Evidence to date, generally suggests that pregnenolone, progesterone, and allopregnanolone may be the most effective pharmacological agents. There is preliminary evidence that pregnenolone mitigates alcohol craving and also improves aspects of both PTSD and AUD, such as HPA axis regulation, state-based anxiety and depressive symptoms. Accordingly, exogenous pregnenolone may improve trauma-related symptoms and AUD and thus warrants further consideration in larger studies.

While exogenous progesterone has not yet been widely studied in the context of PTSD/AUD, its utility to improve negative affect, normalize HPA-axis function and reduce alcohol craving, as well as reduce the use of other substances such as tobacco and cocaine, is promising. However, it should be noted that it has been hypothesized that such positive results are mediated by increases in allopregnanolone levels.[191] Thus, exploration of progesterone's metabolites, including allopregnanolone, is also warranted. This is especially true as exogenous allopregnanolone has also shown significant promise in reducing negative affect as observed in PDD and major depressive disorder. Despite the limited data, exogenous allopregnanolone may mitigate the negative affect observed in trauma-related symptomatology. Similarly, it is likely to decrease alcohol consumption.

The literature also highlights discordant findings across the neurosteroids. Findings often vary between sexes and ages. Nonetheless, SABV has not been systematically examined, as summarized in Tables 1 & 3. Future studies of PTSD and AUD should be designed to explore SABV adequately and must be powered to detect sex differences. This is especially important as there are significant sex differences in PTSD and AUD prevalence and symptoms. Men are more likely to experience a "potentially traumatic event," but women are more likely to develop PTSD after trauma exposure and women are also more vulnerable to consume alcohol in the context of stress.[5,7,20,21] Thus, additional research to explore the potential sexually dimorphic vulnerabilities is needed.

Specifically, we suggest that future research include the "4 C's of Studying Sex," as proposed by the National Institute of Health Office of Research on Women's Health. This guidance advises: 1. a priori *consideration* of sex in the design of a project; 2. *collecting* data based upon sex; 3. *considering* sex using sex-stratified data analytical plans;[192] 4. *communicating* sex-specific findings.[193] Such strategies will permit future sex-based comparisons across the clinical literature and inform treatment development for PTSD/AUD.[193]

It is also difficult to draw conclusions across many of the studies exploring neurosteroids in women, as many of the studies used menstrual cycle subphases, with differing methodologies for categorization. The use of various definitions of menstrual cycle subphases likely also contributes to the incongruous findings. Future studies would benefit from utilizing established best research practices when designing the collection and analysis of hormone levels within a study.[97] This includes studying actual hormone levels as opposed to dichotomized menstrual cycle categorization when possible, as well as consideration of the timing and amplitude of dynamic changes in hormones in premenopausal women. Additionally, studies of neurosteroid fluctuations among women across the female reproductive stages, including pre-, peri- and postmenopause, as well as use of exogenous hormone therapies is important, as these conditions also generate unique changes in neurosteroid levels.[194] Accounting for individual differences[195] and sex differences in the metabolism of progesterone and neurosteroids also may clarify the relationship between hormone levels and PTSD/AUD symptoms as well as response to neurosteroid treatments.

Recent recommendations advocate for rigorous standards to be employed when examining hormone and neurosteroid levels across the menstrual cycle. Specifically, it is important to select a specific menstrual cycle subphase, as well as to choose the most precise identification method available. Current evidence suggests sonographic confirmation is most accurate when identifying menstrual cycle subphases, but a combination of self-reported onset of menses, home urinary LH testing, and confirmatory sex hormone measurement can also be effective in categorizing a subphase. Additionally, it is suggested that methodology be explicitly described and absolute values for each neurosteroid, ratios of neurosteroids, and changes between timepoints also be reported (for complete recommendations see Allen et al., 2016[97]).

Despite the shortcomings in the present body of literature, existing data illustrate the potential for exogenous neurosteroids to attenuate HPA-reactivity, depressive symptoms, and

alcohol craving and consumption, which may effectively treat individuals with co-occurring PTSD and AUD. Surprisingly, despite the high rates of comorbidity among PTSD and AUD, as well as the common vulnerabilities associated with both disorders, neurosteroids do not appear to have been widely studied in concurrent PTSD and AUD. Future studies should explore the effects of endogenous and exogenous neurosteroids in populations with concurrent PTSD and AUD.

7. Conclusion

This review highlights the clinical literature over the past ten years and explores the relationships between neurosteroids, PTSD, and AUD. Recent data supports the idea that neurosteroids can improve trauma-related symptomatology and PTSD, as well as alcohol consumption and alcohol-related variables, such as cravings. Additionally, preliminary evidence supports the possibility that exogenous neurosteroids may be novel treatments, by targeting the underlying mechanism of PTSD/AUD. In particular, pregnenolone, progesterone, and allopregnanolone have shown the most promise in addressing the reciprocal relationship between PTSD and AUD, and thus warrant additional research. It is especially critical to explore such potential interventions among individuals with concurrent PTSD/AUD, as only one study to the authors' knowledge has examined neurosteroids in populations with co-occurring PTSD and AUD.

While these findings are intriguing and offer promise for the development of novel interventions for PTSD and AUD, this review also highlights the inconsistent application of SABV and lack of rigor in assessment of menstrual cycle status and reproductive states in most studies. In particular, consistency in categorizing menstrual cycle status, inclusion of pre- and perimenopausal women, and analysis of exogenous hormonal therapies should be addressed in future studies. Future research should also power studies adequately to address SABV, including stratifying analyses and reporting findings by sex. Such strategies will create homogeneity across studies and provide more rigorous data on the role of neurosteroids in the etiology, maintenance, relapse, and treatment of PTSD and AUD.

References

1. Grant BF, Chou SP, Saha TD, et al. Prevalence of 12-Month Alcohol Use, High-Risk Drinking, and DSM-IV Alcohol Use Disorder in the United States, 2001-2002 to 2012-2013: Results From the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA psychiatry*. 2017;74(9):911-923. doi:10.1001/jamapsychiatry.2017.2161

2. Grant BF, Goldstein RB, Saha TD, et al. Epidemiology of DSM-5 Alcohol Use Disorder: Results From the National Epidemiologic Survey on Alcohol and Related Conditions III. *JAMA psychiatry*. 2015;72(8):757-766. doi:10.1001/jamapsychiatry.2015.0584

3. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. Oct 17 2020;396(10258):1223-1249. doi:10.1016/s0140-6736(20)30752-2

4. NIAAA. Alcohol Use Disorder (AUD) in the United States: Age Groups and Demographic Characteristics. *Alcohol Facts and Statistics*. 2023;

5. Goldstein RB, Smith SM, Chou SP, et al. The epidemiology of DSM-5 posttraumatic stress disorder in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions-III. *Social psychiatry and psychiatric epidemiology*. 2016;51(8):1137-1148. doi:10.1007/s00127-016-1208-5

6. Castillo-Carniglia A, Keyes KM, Hasin DS, Cerdá M. Psychiatric comorbidities in alcohol use disorder. *Lancet Psychiatry*. Dec 2019;6(12):1068-1080. doi:10.1016/s2215-0366(19)30222-6

7. Pietrzak RH, Goldstein RB, Southwick SM, Grant BF. Prevalence and Axis I comorbidity of full and partial posttraumatic stress disorder in the United States: results from Wave 2 of the National Epidemiologic Survey on Alcohol and Related Conditions. *Journal of anxiety disorders*. 2011;25(3):456-465. doi:10.1016/j.janxdis.2010.11.010

8. Simpson TL, Rise P, Browne KC, Lehavot K, Kaysen D. Clinical presentations, social functioning, and treatment receipt among individuals with comorbid life-time PTSD and alcohol use disorders versus drug use disorders: findings from NESARC-III. *Addiction*. Jun 2019;114(6):983-993. doi:10.1111/add.14565

9. Kaysen D, Simpson T, Dillworth T, Larimer ME, Gutner C, Resick PA. Alcohol problems and posttraumatic stress disorder in female crime victims. *J Trauma Stress*. Jun 2006;19(3):399-403. doi:10.1002/jts.20122

10. Back SE, Brady KT, Sonne SC, Verduin ML. Symptom improvement in co-occurring PTSD and alcohol dependence. *J Nerv Ment Dis*. Sep 2006;194(9):690-6. doi:10.1097/01.nmd.0000235794.12794.8a

11. Blanco C, Xu Y, Brady K, Pérez-Fuentes G, Okuda M, Wang S. Comorbidity of posttraumatic stress disorder with alcohol dependence among US adults: results from National Epidemiological Survey on Alcohol and Related Conditions. *Drug and alcohol dependence*. 2013;132(3):630-638. doi:10.1016/j.drugalcdep.2013.04.016

12. North CS, Ringwalt CL, Downs D, Derzon J, Galvin D. Postdisaster course of alcohol use disorders in systematically studied survivors of 10 disasters. *Arch Gen Psychiatry*. Feb 2011;68(2):173-80. doi:10.1001/archgenpsychiatry.2010.131

13. Tripp JC, Worley MJ, Straus E, Angkaw AC, Trim RS, Norman SB. Bidirectional relationship of posttraumatic stress disorder (PTSD) symptom severity and alcohol use over the course of integrated treatment. *Psychol Addict Behav*. Jun 2020;34(4):506-511. doi:10.1037/adb0000564

14. Langdon KJ, Fox AB, King LA, King DW, Eisen S, Vogt D. Examination of the dynamic interplay between posttraumatic stress symptoms and alcohol misuse among combat-exposed Operation Enduring Freedom (OEF)/Operation Iraqi Freedom (OIF) Veterans. *J Affect Disord*. May 15 2016;196:234-42. doi:10.1016/j.jad.2016.02.048

15. Norman SB, Haller M, Hamblen JL, Southwick SM, Pietrzak RH. The burden of co-occurring alcohol use disorder and PTSD in U.S. Military veterans: Comorbidities, functioning, and suicidality. *Psychology of Addictive Behaviors*. 2018;32(2):224-229. doi:10.1037/adb0000348

16. Simpson TL, Hawrilenko M, Goldberg S, Browne K, Lehavot K, Borowitz M. Treatment receipt patterns among individuals with co-occurring posttraumatic stress disorder (PTSD) and substance use disorders. *J Consult Clin Psychol*. Nov 2020;88(11):1039-1051. doi:10.1037/ccp0000600

17. Roberts NP, Roberts PA, Jones N, Bisson JI. Psychological interventions for post-traumatic stress disorder and comorbid substance use disorder: A systematic review and meta-analysis. *Clinical psychology review*. 2015;38:25-38.

18. Petrakis IL, Simpson TL. Posttraumatic Stress Disorder and Alcohol Use Disorder: A Critical Review of Pharmacologic Treatments. *Alcoholism: Clinical and Experimental Research*. 2017/02/01 2017;41(2):226-237. doi:https://doi.org/10.1111/acer.13297

19. White AM. Gender Differences in the Epidemiology of Alcohol Use and Related Harms in the United States. *Alcohol Res*. 2020;40(2):01. doi:10.35946/arcr.v40.2.01

20. Peltier MR, Verplaetse TL, Mineur YS, et al. Sex differences in stress-related alcohol use. *Neurobiology of Stress*. 2019/02/08/ 2019:100149. doi:https://doi.org/10.1016/j.ynstr.2019.100149

21. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. *Psychol Bull*. Nov 2006;132(6):959-92. doi:10.1037/0033-2909.132.6.959

22. Lehavot K, Stappenbeck CA, Luterek JA, Kaysen D, Simpson TL. Gender differences in relationships among PTSD severity, drinking motives, and alcohol use in a comorbid alcohol dependence and PTSD sample. *Psychol Addict Behav*. 2014;28(1):42-52. doi:10.1037/a0032266

23. Peltier MR, Verplaetse TL, Mineur YS, et al. Sex differences in progestogen- and androgen-derived neurosteroids in vulnerability to alcohol and stress-related disorders.

Neuropharmacology. 2021/04/01/ 2021;187:108499. doi:https://doi.org/10.1016/j.neuropharm.2021.108499

24. Morrow AL. Recent developments in the significance and therapeutic relevance of neuroactive steroids--Introduction to the special issue. *Pharmacol Ther*. Oct 2007;116(1):1-6. doi:10.1016/j.pharmthera.2007.04.003

25. Reddy DS. Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Prog Brain Res*. 2010;186:113-37. doi:10.1016/b978-0-444-53630-3.00008-7

26. Reddy DS. Pharmacology of endogenous neuroactive steroids. *Crit Rev Neurobiol*. 2003;15(3-4):197-234. doi:10.1615/critrevneurobiol.v15.i34.20

27. Vallée M. Neurosteroids and potential therapeutics: Focus on pregnenolone. *The Journal of Steroid Biochemistry and Molecular Biology*. 2016/06/01/ 2016;160:78-87. doi:https://doi.org/10.1016/j.jsbmb.2015.09.030

28. Schiller CE, Johnson SL, Abate AC, Schmidt PJ, Rubinow DR. Reproductive Steroid Regulation of Mood and Behavior. *Compr Physiol*. Jun 13 2016;6(3):1135-60. doi:10.1002/cphy.c150014

29. Tomaselli G, Vallée M. Stress and drug abuse-related disorders: The promising therapeutic value of neurosteroids focus on pregnenolone-progesterone-allopregnanolone pathway. *Front Neuroendocrinol*. Oct 2019;55:100789. doi:10.1016/j.yfrne.2019.100789

30. Rasmusson AM, Marx CE, Pineles SL, et al. Neuroactive steroids and PTSD treatment. *Neurosci Lett*. May 10 2017;649:156-163. doi:10.1016/j.neulet.2017.01.054

31. Reddy DS. Neurosteroids: endogenous role in the human brain and therapeutic potentials. *Progress in brain research*. 2010;186:113-137. doi:10.1016/B978-0-444-53630-3.00008-7

32. Verplaetse TL, McKee SA, Petrakis IL. Pharmacotherapy for Co-Occurring Alcohol Use Disorder and Post-Traumatic Stress Disorder: Targeting the Opioidergic, Noradrenergic, Serotonergic, and GABAergic/Glutamatergic Systems. *Alcohol Res*. 2018;39(2):193-205.

33. Weng J-H, Chung B-c. Nongenomic actions of neurosteroid pregnenolone and its metabolites. *Steroids*. 2016/07/01/ 2016;111:54-59. doi:https://doi.org/10.1016/j.steroids.2016.01.017

34. Marx CE, Bradford DW, Hamer RM, et al. Pregnenolone as a novel therapeutic candidate in schizophrenia: emerging preclinical and clinical evidence. *Neuroscience*. 2011/09/15/ 2011;191:78-90. doi:https://doi.org/10.1016/j.neuroscience.2011.06.076

35. Wittliff JL, Andres SA. Progesterone and Progestin Mimics. In: Wexler P, ed. *Encyclopedia of Toxicology (Third Edition)*. Academic Press; 2014:1073-1076.

36. Kimball A, Dichtel LE, Nyer MB, et al. The allopregnanolone to progesterone ratio across the menstrual cycle and in menopause. *Psychoneuroendocrinology*. Feb 2020;112:104512. doi:10.1016/j.psyneuen.2019.104512

37. Kancheva R, Hill M, Cibula D, et al. Relationships of circulating pregnanolone isomers and their polar conjugates to the status of sex, menstrual cycle, and pregnancy. *Journal of Endocrinology*. 01 Oct. 2007 2007;195(1):67-78. doi:10.1677/JOE-06-0192

38. Paul SM, Pinna G, Guidotti A. Allopregnanolone: From molecular pathophysiology to therapeutics. A historical perspective. *Neurobiol Stress*. May 2020;12:100215. doi:10.1016/j.ynstr.2020.100215

39. Lynch WJ, Sofuoglu M. Role of Progesterone in Nicotine Addiction: Evidence From Initiation to Relapse. *Experimental and clinical psychopharmacology*. 2010;18(6):451-461. doi:10.1037/a0021265

40. Giatti S, Diviccaro S, Falvo E, Garcia-Segura LM, Melcangi RC. Physiopathological role of the enzymatic complex 5α-reductase and 3α/β-hydroxysteroid oxidoreductase in the generation of progesterone and testosterone neuroactive metabolites. *Frontiers in Neuroendocrinology*. 2020/04/01/ 2020;57:100836. doi:https://doi.org/10.1016/j.yfrne.2020.100836

41. Peltier MR, Sofuoglu M. Role of Exogenous Progesterone in the Treatment of Men and Women with Substance Use Disorders: A Narrative Review. *CNS drugs*. 2018;32(5):421-435. doi:10.1007/s40263-018-0525-5

42. MacKenzie G, Maguire J. Neurosteroids and GABAergic signaling in health and disease. *Biomol Concepts*. Feb 2013;4(1):29-42. doi:10.1515/bmc-2012-0033

43. Maguire J, Mody I. Steroid hormone fluctuations and GABA(A)R plasticity. *Psychoneuroendocrinology*. Dec 2009;34 Suppl 1(Suppl 1):S84-90. doi:10.1016/j.psyneuen.2009.06.019

44. Girdler SS, Klatzkin R. Neurosteroids in the context of stress: Implications for depressive disorders. *Pharmacology & Therapeutics*. 2007/10/01/ 2007;116(1):125-139. doi:https://doi.org/10.1016/j.pharmthera.2007.05.006

45. Almeida FB, Pinna G, Barros HMT. The Role of HPA Axis and Allopregnanolone on the Neurobiology of Major Depressive Disorders and PTSD. *Int J Mol Sci*. May 23 2021;22(11)doi:10.3390/ijms22115495

46. Gunn BG, Brown AR, Lambert JJ, Belelli D. Neurosteroids and GABA(A) Receptor Interactions: A Focus on Stress. *Front Neurosci*. 2011;5:131. doi:10.3389/fnins.2011.00131

47. Reddy DS. Mass spectrometric assay and physiological-pharmacological activity of androgenic neurosteroids. *Neurochem Int*. Mar-Apr 2008;52(4-5):541-53. doi:10.1016/j.neuint.2007.05.019

48. Reichmann F, Holzer P. Neuropeptide Y: A stressful review. *Neuropeptides*. Feb 2016;55:99-109. doi:10.1016/j.npep.2015.09.008

49. Zukowska-Grojec Z. Neuropeptide Y: a novel sympathetic stress hormone and more. 1995;

50. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med*. Mar 2013;19(3):197-209. doi:10.1016/j.molmed.2012.12.007

51. Lebron-Milad K, Milad MR. Sex differences, gonadal hormones and the fear extinction network: implications for anxiety disorders. *Biology of Mood & Anxiety Disorders*. 2012/02/07 2012;2(1):3. doi:10.1186/2045-5380-2-3

52. Rybaczyk LA, Bashaw MJ, Pathak DR, Moody SM, Gilders RM, Holzschu DL. An overlooked connection: serotonergic mediation of estrogen-related physiology and pathology. *BMC Womens Health*. Dec 20 2005;5:12. doi:10.1186/1472-6874-5-12

53. Becker JB. Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. *Synapse*. 1990;5(2):157-64. doi:10.1002/syn.890050211

54. Yoest KE, Cummings JA, Becker JB. Estradiol, dopamine and motivation. *Cent Nerv Syst Agents Med Chem*. 2014;14(2):83-9. doi:10.2174/1871524914666141226103135

55. Rainey WE, Nakamura Y. Regulation of the adrenal androgen biosynthesis. *J Steroid Biochem Mol Biol*. Feb 2008;108(3-5):281-6. doi:10.1016/j.jsbmb.2007.09.015

56. Schüle C, Eser D, Baghai TC, Nothdurfter C, Kessler JS, Rupprecht R. Neuroactive steroids in affective disorders: target for novel antidepressant or anxiolytic drugs? *Neuroscience*. 2011/09/15/ 2011;191:55-77. doi:https://doi.org/10.1016/j.neuroscience.2011.03.025

57. Dubrovsky B. Neurosteroids, neuroactive steroids, and symptoms of affective disorders. *Pharmacology Biochemistry and Behavior*. 2006/08/01/ 2006;84(4):644-655. doi:https://doi.org/10.1016/j.pbb.2006.06.016

58. Park-Chung M, Malayev A, Purdy RH, Gibbs TT, Farb DH. Sulfated and unsulfated steroids modulate gamma-aminobutyric acidA receptor function through distinct sites. *Brain Res*. May 29 1999;830(1):72-87. doi:10.1016/s0006-8993(99)01381-5

59. Kinzel P, Marx CE, Sollmann N, et al. Serum Neurosteroid Levels Are Associated With Cortical Thickness in Individuals Diagnosed With Posttraumatic Stress Disorder and History of Mild Traumatic Brain Injury. *Clinical EEG and Neuroscience*. 2020/07/01 2020;51(4):285-299. doi:10.1177/1550059420909676

60. Marx CE, Naylor JC, Kilts JD, et al. *Neurosteroids and Traumatic Brain Injury: Translating Biomarkers to Therapeutics; Overview and Pilot Investigations in Iraq and Afghanistan Era Veterans*

Translational Research in Traumatic Brain Injury. © 2016 by Taylor & Francis Group, LLC.; 2016.

61. Umminger LF, Rojczyk P, Seitz-Holland J, et al. White Matter Microstructure Is Associated with Serum Neuroactive Steroids and Psychological Functioning. *Journal of Neurotrauma*. 2023;

62. 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*. 2019/01/01 2019;3:2470547019838570. doi:10.1177/2470547019838570

63. Nillni YI, Pineles SL, Patton SC, Rouse MH, Sawyer AT, Rasmusson AM. Menstrual Cycle Effects on Psychological Symptoms in Women With PTSD. https://doi.org/10.1002/jts.21984. *Journal of Traumatic Stress*. 2015/02/01 2015;28(1):1-7. doi:https://doi.org/10.1002/jts.21984

64. Nillni YI, Arditte Hall KA, Langdon KJ, Pineles SL. Examination of the stability of the anxiety sensitivity index across the menstrual cycle in trauma-exposed women with and without PTSD. *Anxiety Stress Coping*. Jan 2020;33(1):115-121. doi:10.1080/10615806.2019.1660322

65. Lalonde CS, Mekawi Y, Ethun KF, et al. Sex differences in peritraumatic inflammatory cytokines and steroid hormones contribute to prospective risk for nonremitting posttraumatic stress disorder. *Chronic Stress*. 2021;5:24705470211032208.

66. Krinke E, Held U, Steigmiller K, Felmingham K, Kleim B. Sex hormones and cortisol during experimental trauma memory consolidation: Prospective association with intrusive memories. *European Journal of Psychotraumatology*. 2022;13(1):2040818.

67. Hennessy VE, Troebinger L, Iskandar G, Das RK, Kamboj SK. Accelerated forgetting of a trauma-like event in healthy men and women after a single dose of hydrocortisone. *Translational Psychiatry*. 2022/08/31 2022;12(1):354. doi:10.1038/s41398-022-02126-2

68. Inslicht SS, Richards A, Madden E, et al. Sex differences in neurosteroid and hormonal responses to metyrapone in posttraumatic stress disorder. *Psychopharmacology*. 2014/09/01 2014;231(17):3581-3595. doi:10.1007/s00213-014-3621-3

69. Juster R-P, Raymond C, Desrochers AB, et al. Sex hormones adjust “sex-specific” reactive and diurnal cortisol profiles. *Psychoneuroendocrinology*. 2016/01/01/ 2016;63:282-290. doi:https://doi.org/10.1016/j.psyneuen.2015.10.012

70. Childs E, Van Dam NT, de Wit H. Effects of acute progesterone administration upon responses to acute psychosocial stress in men. *Exp Clin Psychopharmacol*. Feb 2010;18(1):78-86. doi:10.1037/a0018060

71. Stephens MA, Mahon PB, McCaul ME, Wand GS. Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology*. Apr 2016;66:47-55. doi:10.1016/j.psyneuen.2015.12.021

72. Kim BK, Fonda JR, Hauger RL, et al. Composite contributions of cerebrospinal fluid GABAergic neurosteroids, neuropeptide Y and interleukin-6 to PTSD symptom severity in men with PTSD. *Neurobiol Stress*. May 2020;12:100220. doi:10.1016/j.ynstr.2020.100220

73. Wegerer M, Kerschbaum H, Blechert J, Wilhelm FH. Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. *Neurobiology of Learning and Memory*. 2014/12/01/ 2014;116:145-154. doi:https://doi.org/10.1016/j.nlm.2014.10.001

74. Pineles SL, Blumenthal TD, Curreri AJ, et al. Prepulse inhibition deficits in women with PTSD. *Psychophysiology*. Sep 2016;53(9):1377-85. doi:10.1111/psyp.12679

75. Girdler SS, Klatzkin R. Neurosteroids in the context of stress: implications for depressive disorders. *Pharmacol Ther*. Oct 2007;116(1):125-39. doi:10.1016/j.pharmthera.2007.05.006

76. Schüle C, Nothdurfter C, Rupprecht R. The role of allopregnanolone in depression and anxiety. *Progress in Neurobiology*. 2014/02/01/ 2014;113:79-87. doi:https://doi.org/10.1016/j.pneurobio.2013.09.003

77. Rauch SAM, Sripada R, Burton M, et al. Neuroendocrine biomarkers of prolonged exposure treatment response in military-related PTSD. *Psychoneuroendocrinology*. 2020/09/01/ 2020;119:104749. doi:https://doi.org/10.1016/j.psyneuen.2020.104749

78. Kringelbach ML. The human orbitofrontal cortex: linking reward to hedonic experience. *Nature Reviews Neuroscience*. 2005/09/01 2005;6(9):691-702. doi:10.1038/nrn1747

79. Rasmusson AM, King MW, Valovski I, et al. Relationships between cerebrospinal fluid GABAergic neurosteroid levels and symptom severity in men with PTSD. *Psychoneuroendocrinology*. Apr 2019;102:95-104. doi:10.1016/j.psyneuen.2018.11.027

80. Pineles SL, Nillni YI, Pinna G, et al. PTSD in women is associated with a block in conversion of progesterone to the GABAergic neurosteroids allopregnanolone and pregnanolone measured in plasma. *Psychoneuroendocrinology*. Jul 2018;93:133-141. doi:10.1016/j.psyneuen.2018.04.024

81. Pineles SL, Nillni YI, Pinna G, et al. Associations between PTSD-Related extinction retention deficits in women and plasma steroids that modulate brain GABAA and NMDA receptor activity. *Neurobiology of Stress*. 2020/11/01/ 2020;13:100225. doi:https://doi.org/10.1016/j.ynstr.2020.100225

82. Mendoza C, Barreto GE, Ávila-Rodriguez M, Echeverria V. Role of neuroinflammation and sex hormones in war-related PTSD. *Molecular and Cellular Endocrinology*. 2016/10/15/ 2016;434:266-277. doi:https://doi.org/10.1016/j.mce.2016.05.016

83. Reijnen A, Geuze E, Vermetten E. The effect of deployment to a combat zone on testosterone levels and the association with the development of posttraumatic stress symptoms: A longitudinal prospective Dutch military cohort study. *Psychoneuroendocrinology*. 2015/01/01/ 2015;51:525-533. doi:https://doi.org/10.1016/j.psyneuen.2014.07.017

84. Feklicheva I, Boks MP, de Kloet ER, et al. Biomarkers in PTSD-susceptible and resistant veterans with war experience of more than ten years ago: FOCUS ON cortisol, thyroid hormones, testosterone and GABA. *Journal of Psychiatric Research*. 2022/04/01/ 2022;148:258-263. doi:https://doi.org/10.1016/j.jpsychires.2021.11.032

85. Lehrner A, Flory JD, Bierer LM, Makotkine I, Marmar CR, Yehuda R. Sexual dysfunction and neuroendocrine correlates of posttraumatic stress disorder in combat veterans: Preliminary findings. *Psychoneuroendocrinology*. 2016/01/01/ 2016;63:271-275. doi:https://doi.org/10.1016/j.psyneuen.2015.10.015

86. Farina EK, Taylor JC, Means GE, et al. Effects of Combat Deployment on Anthropometrics and Physiological Status of U.S. Army Special Operations Forces Soldiers. *Military Medicine*. 2017;182(3-4):e1659-e1668. doi:10.7205/MILMED-D-16-00022

87. Josephs RA, Cobb AR, Lancaster CL, Lee H-J, Telch MJ. Dual-hormone stress reactivity predicts downstream war-zone stress-evoked PTSD. *Psychoneuroendocrinology*. 2017/04/01/ 2017;78:76-84. doi:https://doi.org/10.1016/j.psyneuen.2017.01.013

88. Cobb AR, Josephs RA, Lancaster CL, Lee H-J, Telch MJ. Cortisol, Testosterone, and Prospective Risk for War-zone Stress-Evoked Depression. *Military Medicine*. 2018;183(11-12):e535-e545. doi:10.1093/milmed/usy065

89. Deuter CE, Duesenberg M, Hellmann-Regen J, et al. Psychosocial stress increases testosterone in patients with borderline personality disorder, post-traumatic stress disorder and healthy participants. *Borderline Personality Disorder and Emotion Dysregulation*. 2021/02/01 2021;8(1):3. doi:10.1186/s40479-021-00145-x

90. Karlović D, Serretti A, Marčinko D, Martinac M, Silić A, Katinić K. Serum testosterone concentration in combat-related chronic posttraumatic stress disorder. *Neuropsychobiology*. 2012;65(2):90-95.

91. Rieder JK, Kleshchova O, Weierich MR. Estradiol, stress reactivity, and daily affective experiences in trauma-exposed women. *Psychol Trauma*. Jul 2022;14(5):738-746. doi:10.1037/tra0001113

92. Hack LM, Nishitani S, Knight AK, et al. Epigenetic prediction of 17β-estradiol and relationship to trauma-related outcomes in women. *Comprehensive Psychoneuroendocrinology*. 2021/05/01/ 2021;6:100045. doi:https://doi.org/10.1016/j.cpnec.2021.100045

93. Sartin-Tarm A, Ross MC, Privatsky AA, Cisler JM. Estradiol Modulates Neural and Behavioral Arousal in Women With Posttraumatic Stress Disorder During a Fear Learning and Extinction Task. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*. 2020/12/01/ 2020;5(12):1114-1122. doi:https://doi.org/10.1016/j.bpsc.2020.04.012

94. Rieder JK, Kleshchova O, Weierich MR. Trauma Characteristics Moderate the Relation Between Estradiol and Trauma-Related Symptoms. *J Trauma Stress*. Feb 2022;35(1):246-256. doi:10.1002/jts.22725

95. Glover EM, Jovanovic T, Mercer KB, et al. Estrogen Levels Are Associated with Extinction Deficits in Women with Posttraumatic Stress Disorder. *Biological Psychiatry*. 2012/07/01/ 2012;72(1):19-24. doi:https://doi.org/10.1016/j.biopsych.2012.02.031

96. Glover EM, Mercer KB, Norrholm SD, et al. Inhibition of fear is differentially associated with cycling estrogen levels in women. *J Psychiatry Neurosci*. Sep 2013;38(5):341-8. doi:10.1503/jpn.120129

97. Allen AM, McRae-Clark AL, Carlson S, et al. Determining menstrual phase in human biobehavioral research: A review with recommendations. *Exp Clin Psychopharmacol*. Feb 2016;24(1):1-11. doi:10.1037/pha0000057

98. Pineles SL, Nillni YI, King MW, et al. Extinction retention and the menstrual cycle: Different associations for women with posttraumatic stress disorder. *J Abnorm Psychol*. Apr 2016;125(3):349-55. doi:10.1037/abn0000138

99. Soni M, Curran VH, Kamboj SK. Identification of a narrow post-ovulatory window of vulnerability to distressing involuntary memories in healthy women. *Neurobiology of Learning and Memory*. 2013/09/01/ 2013;104:32-38. doi:https://doi.org/10.1016/j.nlm.2013.04.003

100. Franke LK, Miedl SF, Danböck SK, et al. Estradiol during (analogue-)trauma: Risk- or protective factor for intrusive re-experiencing? *Psychoneuroendocrinology*. 2022/09/01/ 2022;143:105819. doi:https://doi.org/10.1016/j.psyneuen.2022.105819

101. Bissière S, Plachta N, Hoyer D, et al. The rostral anterior cingulate cortex modulates the efficiency of amygdala-dependent fear learning. *Biol Psychiatry*. May 1 2008;63(9):821-31. doi:10.1016/j.biopsych.2007.10.022

102. Koenigs M, Grafman J. Posttraumatic stress disorder: the role of medial prefrontal cortex and amygdala. *Neuroscientist*. Oct 2009;15(5):540-8. doi:10.1177/1073858409333072

103. Miedl SF, Wegerer M, Kerschbaum H, Blechert J, Wilhelm FH. Neural activity during traumatic film viewing is linked to endogenous estradiol and hormonal contraception. *Psychoneuroendocrinology*. 2018/01/01/ 2018;87:20-26. doi:https://doi.org/10.1016/j.psyneuen.2017.10.006

104. Maeng LY, Milad MR. Sex differences in anxiety disorders: Interactions between fear, stress, and gonadal hormones. *Horm Behav*. Nov 2015;76:106-17. doi:10.1016/j.yhbeh.2015.04.002

105. Ferree NK, Wheeler M, Cahill L. The influence of emergency contraception on post-traumatic stress symptoms following sexual assault. *J Forensic Nurs*. Sep 2012;8(3):122-30. doi:10.1111/j.1939-3938.2012.01134.x

106. Bartholomew ME, Rozalski V, Richards A, et al. Impact of hormonal contraceptives on sex differences in fear conditioning and fear extinction in PTSD. *Learn Mem*. Sep 2022;29(9):332-339. doi:10.1101/lm.053597.122

107. Graham BM, Milad MR. Blockade of Estrogen by Hormonal Contraceptives Impairs Fear Extinction in Female Rats and Women. *Biological Psychiatry*. 2013/02/15/ 2013;73(4):371-378. doi:https://doi.org/10.1016/j.biopsych.2012.09.018

108. Antov MI, Stockhorst U. Stress exposure prior to fear acquisition interacts with estradiol status to alter recall of fear extinction in humans. *Psychoneuroendocrinology*. Nov 2014;49:106-18. doi:10.1016/j.psyneuen.2014.06.022

109. van Zuiden M, Haverkort SQ, Tan Z, Daams J, Lok A, Olff M. DHEA and DHEA-S levels in posttraumatic stress disorder: A meta-analytic review. *Psychoneuroendocrinology*. 2017/10/01/ 2017;84:76-82. doi:https://doi.org/10.1016/j.psyneuen.2017.06.010

110. Jergović M, Bendelja K, Savić Mlakar A, et al. Circulating levels of hormones, lipids, and immune mediators in post-traumatic stress disorder - a 3-month follow-up study. *Front Psychiatry*. 2015;6:49. doi:10.3389/fpsyt.2015.00049

111. Orta OR, Huang T, Kubzansky LD, et al. The association between abuse history in childhood and salivary rhythms of cortisol and DHEA in postmenopausal women. *Psychoneuroendocrinology*. 2020/02/01/ 2020;112:104515. doi:https://doi.org/10.1016/j.psyneuen.2019.104515

112. Mouthaan J, Sijbrandij M, Luitse JSK, Goslings JC, Gersons BPR, Olff M. The role of acute cortisol and DHEAS in predicting acute and chronic PTSD symptoms. *Psychoneuroendocrinology*. 2014/07/01/ 2014;45:179-186. doi:https://doi.org/10.1016/j.psyneuen.2014.04.001

113. Usta MB, Tuncel OK, Akbas S, Aydin B, Say GN. Decreased dehydroepiandrosterone sulphate levels in adolescents with post-traumatic stress disorder after single sexual trauma. *Nord J Psychiatry*. 2016;70(2):116-20. doi:10.3109/08039488.2015.1056752

114. Suurd Ralph C, Vartanian O, Lieberman HR, Morgan CA, Cheung B. The effects of captivity survival training on mood, dissociation, PTSD symptoms, cognitive performance and stress hormones. *International Journal of Psychophysiology*. 2017/07/01/ 2017;117:37-47. doi:https://doi.org/10.1016/j.ijpsycho.2017.04.002

115. Van Voorhees EE, Dennis MF, Calhoun PS, Beckham JC. Association of DHEA, DHEAS, and cortisol with childhood trauma exposure and post-traumatic stress disorder. *Int Clin Psychopharmacol*. Jan 2014;29(1):56-62. doi:10.1097/YIC.0b013e328364ecd1

116. Van Voorhees EE, Dennis MF, McClernon FJ, Calhoun PS, Buse NA, Beckham JC. The association of dehydroepiandrosterone and dehydroepiandrosterone sulfate with anxiety sensitivity and electronic diary negative affect among smokers with and without posttraumatic stress disorder. *J Clin Psychopharmacol*. Aug 2013;33(4):556-60. doi:10.1097/JCP.0b013e3182968962

117. Blasco-Ros C, Herbert J, Martinez M. Different profiles of mental and physical health and stress hormone response in women victims of intimate partner violence. *Journal of Acute Disease*. 2014;3(4):303-313.

118. Bicanic IA, Postma RM, Sinnema G, et al. Salivary cortisol and dehydroepiandrosterone sulfate in adolescent rape victims with post traumatic stress disorder. *Psychoneuroendocrinology*. Mar 2013;38(3):408-15. doi:10.1016/j.psyneuen.2012.06.015

119. Golier JA, Caramanica K, Makotkine I, Sher L, Yehuda R. Cortisol response to cosyntropin administration in military veterans with or without posttraumatic stress disorder. *Psychoneuroendocrinology*. 2014/02/01/ 2014;40:151-158. doi:https://doi.org/10.1016/j.psyneuen.2013.10.020

120. Usta MB, Gumus YY, Say GN, Bozkurt A, Şahin B, Karabekiroğlu K. Basal blood DHEA-S/cortisol levels predicts EMDR treatment response in adolescents with PTSD. *Nord J Psychiatry*. Apr 2018;72(3):164-172. doi:10.1080/08039488.2017.1406984

121. Nijdam MJ, van Amsterdam JGC, Gersons BPR, Olff M. Dexamethasone-suppressed cortisol awakening response predicts treatment outcome in posttraumatic stress disorder. *Journal of Affective Disorders*. 2015/09/15/ 2015;184:205-208. doi:https://doi.org/10.1016/j.jad.2015.05.058

122. Kim SH, Schneider SM, Bevans M, et al. PTSD Symptom Reduction With Mindfulness-Based Stretching and Deep Breathing Exercise: Randomized Controlled Clinical Trial of Efficacy. *The Journal of Clinical Endocrinology & Metabolism*. 2013;98(7):2984-2992. doi:10.1210/jc.2012-3742

123. Pierucci-Lagha A, Covault J, Feinn R, et al. Subjective effects and changes in steroid hormone concentrations in humans following acute consumption of alcohol. *Psychopharmacology (Berl)*. Jun 2006;186(3):451-61. doi:10.1007/s00213-005-0231-0

124. Kärkkäinen O, Häkkinen MR, Auriola S, Kautiainen H, Tiihonen J, Storvik M. Increased steroid hormone dehydroepiandrosterone and pregnenolone levels in post-mortem brain samples of alcoholics. *Alcohol*. May 2016;52:63-70. doi:10.1016/j.alcohol.2016.03.002

125. Martel MM, Eisenlohr-Moul T, Roberts B. Interactive effects of ovarian steroid hormones on alcohol use and binge drinking across the menstrual cycle. *J Abnorm Psychol*. Nov 2017;126(8):1104-1113. doi:10.1037/abn0000304

126. Holzhauer CG, Wemm SE, Wulfert E, Cao Z. Fluctuations in progesterone moderate the relationship between daily mood and alcohol use in young adult women. *Addictive Behaviors*. 2020/02/01/ 2020;101:106146. doi:https://doi.org/10.1016/j.addbeh.2019.106146

127. Joyce KM, Hudson A, O'Connor R, et al. Changes in coping and social motives for drinking and alcohol consumption across the menstrual cycle. https://doi.org/10.1002/da.22699. *Depression and Anxiety*. 2018/04/01 2018;35(4):313-320. doi:https://doi.org/10.1002/da.22699

128. Weinland C, Mühle C, Kornhuber J, Lenz B. Progesterone serum levels correlate negatively with craving in female postmenopausal in-patients with alcohol use disorder: A sex- and menopausal status-separated study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2021/08/30/ 2021;110:110278. doi:https://doi.org/10.1016/j.pnpbp.2021.110278

129. Hayaki J, Holzhauer CG, Epstein EE, et al. Menstrual cycle phase, alcohol consumption, alcohol cravings, and mood among women in outpatient treatment for alcohol use disorder. *Psychol Addict Behav*. Sep 2020;34(6):680-689. doi:10.1037/adb0000576

130. Tosun NL, Fieberg AM, Eberly LE, et al. Exogenous progesterone for smoking cessation in men and women: a pilot double-blind, placebo-controlled randomized clinical trial. *Addiction (Abingdon, England)*. 2019;114(10):1800-1813. doi:10.1111/add.14645

131. Morrow AL, Boero G, Porcu P. A rationale for allopregnanolone treatment of alcohol use disorders: basic and clinical studies. *Alcoholism: Clinical and Experimental Research*. 2020;44(2):320-339.

132. Gatta E, Camussi D, Auta J, Guidotti A, Pandey SC. Neurosteroids (allopregnanolone) and alcohol use disorder: From mechanisms to potential pharmacotherapy. *Pharmacol Ther*. Dec 2022;240:108299. doi:10.1016/j.pharmthera.2022.108299

133. Hasirci AS, Maldonado-Devincci AM, Beattie MC, O'Buckley TK, Morrow AL. Cellular GABAergic Neuroactive Steroid (3α,5α)-3-Hydroxy-Pregnan-20-One (3α,5α-THP) Immunostaining Levels Are Increased in the Ventral Tegmental Area of Human Alcohol Use

Disorder Patients: A Postmortem Study. *Alcohol Clin Exp Res*. Feb 2017;41(2):299-311. doi:10.1111/acer.13300

134. Gatta E, Guidotti A, Saudagar V, et al. Epigenetic Regulation of GABAergic Neurotransmission and Neurosteroid Biosynthesis in Alcohol Use Disorder. *Int J Neuropsychopharmacol*. Feb 15 2021;24(2):130-141. doi:10.1093/ijnp/pyaa073

135. Nyberg S, Andersson A, Zingmark E, Wahlström G, Bäckström T, Sundström-Poromaa I. The effect of a low dose of alcohol on allopregnanolone serum concentrations across the menstrual cycle in women with severe premenstrual syndrome and controls. *Psychoneuroendocrinology*. Oct 2005;30(9):892-901. doi:10.1016/j.psyneuen.2005.04.016

136. Ray LA, Hutchison KE, Ashenhurst JR, Morrow AL. Naltrexone selectively elevates GABAergic neuroactive steroid levels in heavy drinkers with the Asp40 allele of the OPRM1 gene: a pilot investigation. *Alcohol Clin Exp Res*. Aug 2010;34(8):1479-87. doi:10.1111/j.1530-0277.2010.01233.x

137. Holdstock L, Penland SN, Morrow AL, de Wit H. Moderate doses of ethanol fail to increase plasma levels of neurosteroid 3alpha-hydroxy-5alpha-pregnan-20-one-like immunoreactivity in healthy men and women. *Psychopharmacology (Berl)*. Jun 2006;186(3):442-50. doi:10.1007/s00213-005-0187-0

138. Dorgan JF, Baer DJ, Albert PS, et al. Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J Natl Cancer Inst*. May 2 2001;93(9):710-5. doi:10.1093/jnci/93.9.710

139. Sarkola T, Mäkisalo H, Fukunaga T, Eriksson CJ. Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol Clin Exp Res*. Jun 1999;23(6):976-82.

140. Hirko KA, Spiegelman D, Willett WC, Hankinson SE, Eliassen AH. Alcohol consumption in relation to plasma sex hormones, prolactin, and sex hormone-binding globulin in premenopausal women. *Cancer Epidemiol Biomarkers Prev*. Dec 2014;23(12):2943-53. doi:10.1158/1055-9965.Epi-14-0982

141. Hartman TJ, Sisti JS, Hankinson SE, Xu X, Eliassen AH, Ziegler R. Alcohol Consumption and Urinary Estrogens and Estrogen Metabolites in Premenopausal Women. *Horm Cancer*. Feb 2016;7(1):65-74. doi:10.1007/s12672-015-0249-7

142. Schliep KC, Zarek SM, Schisterman EF, et al. Alcohol intake, reproductive hormones, and menstrual cycle function: a prospective cohort study. *Am J Clin Nutr*. Oct 2015;102(4):933-42. doi:10.3945/ajcn.114.102160

143. Playdon MC, Coburn SB, Moore SC, et al. Alcohol and oestrogen metabolites in postmenopausal women in the Women's Health Initiative Observational Study. *British Journal of Cancer*. 2018/02/01 2018;118(3):448-457. doi:10.1038/bjc.2017.419

144. Warren JG, Goodwin L, Gage SH, Rose AK. The effects of menstrual cycle stage and hormonal contraception on alcohol consumption and craving: A pilot investigation. *Comprehensive Psychoneuroendocrinology*. 2021/02/01/ 2021;5:100022. doi:https://doi.org/10.1016/j.cpnec.2020.100022

145. Griffith AK, Martel MM, Eisenlohr-Moul T, Fillmore MT. Heightened sensitivity to the disinhibiting effect of alcohol in women during the late follicular phase of the menstrual cycle. *Exp Clin Psychopharmacol*. Oct 20 2022;doi:10.1037/pha0000611

146. Vatsalya V, Issa JE, Hommer DW, Ramchandani VA. Pharmacodynamic effects of intravenous alcohol on hepatic and gonadal hormones: influence of age and sex. *Alcohol Clin Exp Res*. Feb 2012;36(2):207-13. doi:10.1111/j.1530-0277.2011.01600.x

147. Jensen TK, Gottschau M, Madsen JO, et al. Habitual alcohol consumption associated with reduced semen quality and changes in reproductive hormones; a cross-sectional study among 1221 young Danish men. *BMJ Open*. Oct 2 2014;4(9):e005462. doi:10.1136/bmjopen-2014-005462

148. de Water E, Braams BR, Crone EA, Peper JS. Pubertal maturation and sex steroids are related to alcohol use in adolescents. *Hormones and Behavior*. 2013/02/01/ 2013;63(2):392-397. doi:https://doi.org/10.1016/j.yhbeh.2012.11.018

149. Tin Tin S, Key TJ, Reeves GK. Alcohol Intake and Endogenous Hormones in Pre- and Postmenopausal Women: Findings from the UK Biobank. *Cancer Epidemiol Biomarkers Prev*. Dec 2021;30(12):2294-2301. doi:10.1158/1055-9965.Epi-21-0789

150. Erol A, Ho AMC, Winham SJ, Karpyak VM. Sex hormones in alcohol consumption: a systematic review of evidence. https://doi.org/10.1111/adb.12589. *Addiction Biology*. 2019/03/01 2019;24(2):157-169. doi:https://doi.org/10.1111/adb.12589

151. Heberlein A, Lenz B, Opfermann B, et al. Association of testosterone and BDNF serum levels with craving during alcohol withdrawal. *Alcohol*. 2016

2016-08-26 2016;54:67-72. doi:https://doi.org/10.1016/j.alcohol.2016.06.004

152. Peters S, Jolles DJ, Duijvenvoorde ACKV, Crone EA, Peper JS. The link between testosterone and amygdala–orbitofrontal cortex connectivity in adolescent alcohol use. *Psychoneuroendocrinology*. 2015/03/01/ 2015;53:117-126. doi:https://doi.org/10.1016/j.psyneuen.2015.01.004

153. Crane NA, Gorka SM, Phan KL, Childs E. Amygdala-orbitofrontal functional connectivity mediates the relationship between sensation seeking and alcohol use among binge-drinking adults. *Drug Alcohol Depend*. Nov 1 2018;192:208-214. doi:10.1016/j.drugalcdep.2018.07.044

154. Braams BR, Peper JS, van der Heide D, Peters S, Crone EA. Nucleus accumbens response to rewards and testosterone levels are related to alcohol use in adolescents and young adults. *Developmental Cognitive Neuroscience*. 2016/02/01/ 2016;17:83-93. doi:https://doi.org/10.1016/j.dcn.2015.12.014

155. Endogenous Hormones and Breast Cancer Collaborative Group KT, Appleby PN, et al. Sex hormones and risk of breast cancer in premenopausal women: a collaborative reanalysis of individual participant data from seven prospective studies. *The Lancet Oncology*. 2013/09/01/ 2013;14(10):1009-1019. doi:https://doi.org/10.1016/S1470-2045(13)70301-2

156. Li N, Fu S, Zhu F, Deng X, Shi X. Alcohol intake induces diminished ovarian reserve in childbearing age women. https://doi.org/10.1111/j.1447-0756.2012.01992.x. *Journal of*

Obstetrics and Gynaecology Research. 2013/02/01 2013;39(2):516-521. doi:https://doi.org/10.1111/j.1447-0756.2012.01992.x

157. Peltier MR, Verplaetse TL, Roberts W, et al. Changes in excessive alcohol use among older women across the menopausal transition: a longitudinal analysis of the Study of Women's Health Across the Nation. *Biology of Sex Differences*. 2020/07/14 2020;11(1):37. doi:10.1186/s13293-020-00314-7

158. Nagaya T, Kondo Y, Okinaka T. Serum dehydroepiandrosterone-sulfate reflects age better than health status, and may increase with cigarette smoking and alcohol drinking in middle-aged men. *Aging Clinical and Experimental Research*. 2012/04/01 2012;24(2):134-138. doi:10.1007/BF03325159

159. Aluja A, Balada F, García O, Aymami N, García LF. `Association study of dehydroepiandrosterone sulfate, impulsivity personality traits and moderate alcohol consumption in men. *Personality and Individual Differences*. 2023/02/01/ 2023;201:111924. doi:https://doi.org/10.1016/j.paid.2022.111924

160. Wemm S, Fanean A, Baker A, Blough ER, Mewaldt S, Bardi M. Problematic drinking and physiological responses among female college students. *Alcohol*. Mar 2013;47(2):149-57. doi:10.1016/j.alcohol.2012.12.006

161. Schrieks IC, Joosten MM, Klöpping-Ketelaars WA, Witkamp RF, Hendriks HF. Moderate alcohol consumption after a mental stressor attenuates the endocrine stress response. *Alcohol*. Dec 2016;57:29-34. doi:10.1016/j.alcohol.2016.10.006

162. Weinland C, Mühle C, von Zimmermann C, Kornhuber J, Lenz B. Sulphated dehydroepiandrosterone serum levels are reduced in women with alcohol use disorder and correlate negatively with craving: A sex-separated cross-sectional and longitudinal study. *Addict Biol*. Mar 2022;27(2):e13135. doi:10.1111/adb.13135

163. Ransome Y, Slopen N, Karlsson O, Williams DR. The association between alcohol abuse and neuroendocrine system dysregulation: Race differences in a National sample. *Brain, Behavior, and Immunity*. 2017/11/01/ 2017;66:313-321. doi:https://doi.org/10.1016/j.bbi.2017.07.154

164. Obasi EM, Tackett JL, Shirtcliff EA, Cavanagh L. The Effects of Alcohol and Cigarette Consumption on Dehydroepiandrosterone (DHEA) in Rural African Americans. *Journal of Black Psychology*. 2017;43(6):588-607. doi:10.1177/0095798416665742

165. Milivojevic V, Sullivan L, Tiber J, et al. Pregnenolone effects on provoked alcohol craving, anxiety, HPA axis, and autonomic arousal in individuals with alcohol use disorder. *Psychopharmacology (Berl)*. Jan 2023;240(1):101-114. doi:10.1007/s00213-022-06278-3

166. Milivojevic V, Charron L, Fogelman N, Hermes G, Sinha R. Pregnenolone Reduces Stress-Induced Craving, Anxiety, and Autonomic Arousal in Individuals with Cocaine Use Disorder. *Biomolecules*. Oct 29 2022;12(11)doi:10.3390/biom12111593

167. Brown ES, Park J, Marx CE, et al. A Randomized, Double-Blind, Placebo-Controlled Trial of Pregnenolone for Bipolar Depression. *Neuropsychopharmacology*. 2014/11/01 2014;39(12):2867-2873. doi:10.1038/npp.2014.138

168. Sripada RK, Marx CE, King AP, Rampton JC, Ho SS, Liberzon I. Allopregnanolone Elevations Following Pregnenolone Administration Are Associated with Enhanced Activation of Emotion Regulation Neurocircuits. *Biological Psychiatry*. 2013/06/01/ 2013;73(11):1045-1053. doi:https://doi.org/10.1016/j.biopsych.2012.12.008

169. Sofuoglu M, Mouratidis M, Mooney M. Progesterone improves cognitive performance and attenuates smoking urges in abstinent smokers. *Psychoneuroendocrinology*. 08/02 2011;36(1):123-132. doi:10.1016/j.psyneuen.2010.07.005

170. Sofuoglu M, Mitchell E, Mooney M. Progesterone effects on subjective and physiological responses to intravenous nicotine in male and female smokers. *Human psychopharmacology*. 2009;24(7):559-564. doi:10.1002/hup.1055

171. Sofuoglu M, Babb DA, Hatsukami DK. Progesterone treatment during the early follicular phase of the menstrual cycle: Effects on smoking behavior in women. *Pharmacology Biochemistry and Behavior*. 2001/05/01/ 2001;69(1):299-304. doi:https://doi.org/10.1016/S0091-3057(01)00527-5

172. Sofuoglu M, Babb DA, Hatsukami DK. Effects of progesterone treatment on smoked cocaine response in women. *Pharmacol Biochem Behav*. May 2002;72(1-2):431-5. doi:10.1016/s0091-3057(02)00716-5

173. Evans SM, Foltin RW. Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacology*. Mar 2006;31(3):659-74. doi:10.1038/sj.npp.1300887

174. Fox HC, Sofuoglu M, Morgan PT, Tuit KL, Sinha R. The effects of exogenous progesterone on drug craving and stress arousal in cocaine dependence: Impact of gender and cue type. *Psychoneuroendocrinology*. 01/29 2013;38(9):1532-1544. doi:10.1016/j.psyneuen.2012.12.022

175. Yonkers KA, Forray A, Nich C, et al. Progesterone Reduces Cocaine Use in Postpartum Women with a Cocaine Use Disorder: A Randomized,Double-Blind Study. *Lancet Psychiatry*. Oct 1 2014;1(5):360-367. doi:10.1016/s2215-0366(14)70333-5

176. Allen SS, Allen AM, Lunos S, Tosun N. Progesterone and Postpartum Smoking Relapse: A Pilot Double-Blind Placebo-Controlled Randomized Trial. *Nicotine Tob Res*. Nov 2016;18(11):2145-2153. doi:10.1093/ntr/ntw156

177. Forray A, Gilstad-Hayden K, Suppies C, Bogen D, Sofuoglu M, Yonkers KA. Progesterone for smoking relapse prevention following delivery: A pilot, randomized, double-blind study. *Psychoneuroendocrinology*. Dec 2017;86:96-103. doi:10.1016/j.psyneuen.2017.09.012

178. Milivojevic V, Sinha R, Morgan PT, Sofuoglu M, Fox HC. Effects of endogenous and exogenous progesterone on emotional intelligence in cocaine dependent men and women who also abuse alcohol. *Human psychopharmacology*. 11/03 2014;29(6):589-598. doi:10.1002/hup.2446

179. Reed SC, Levin FR, Evans SM. The effects of progesterone pretreatment on the response to oral d-amphetamine in Women. *Horm Behav*. Aug 2010;58(3):533-43. doi:10.1016/j.yhbeh.2010.04.003

180. Le Mellédo J-M, Jhangri GS, Lott P, et al. Effect of medroxyprogesterone pretreatment on pentagastrin-induced panic symptoms in females with panic disorder. *Psychiatry Research*. 2001/04/15/ 2001;101(3):237-242. doi:https://doi.org/10.1016/S0165-1781(01)00233-5

181. Ralevski E, Newcomb, J., Pisani, E., DeNegre, D., Peltier, M.R., Jane, J., Yoon, G., Petrakis, I.L. Progesterone attenuates the stress response in individuals with alcohol dependence and post-traumatic stress disorder.

182. Möller AT, Bäckström T, Nyberg S, Söndergaard HP, Helström L. Women with PTSD have a changed sensitivity to GABA-A receptor active substances. *Psychopharmacology*. 2016/06/01 2016;233(11):2025-2033. doi:10.1007/s00213-014-3776-y

183. Kanes S, Colquhoun H, Gunduz-Bruce H, et al. Brexanolone (SAGE-547 injection) in post-partum depression: a randomised controlled trial. *The Lancet*. 2017/07/29/ 2017;390(10093):480-489. doi:https://doi.org/10.1016/S0140-6736(17)31264-3

184. Meltzer-Brody S, Colquhoun H, Riesenberg R, et al. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet*. Sep 22 2018;392(10152):1058-1070. doi:10.1016/s0140-6736(18)31551-4

185. Deligiannidis KM, Meltzer-Brody S, Gunduz-Bruce H, et al. Effect of Zuranolone vs Placebo in Postpartum Depression: A Randomized Clinical Trial. *JAMA Psychiatry*. 2021;78(9):951-959. doi:10.1001/jamapsychiatry.2021.1559

186. Clayton AH, Lasser R, Nandy I, Sankoh AJ, Jonas J, Kanes SJ. Zuranolone in Major Depressive Disorder: Results From MOUNTAIN-A Phase 3, Multicenter, Double-Blind, Randomized, Placebo-Controlled Trial. *J Clin Psychiatry*. Feb 20 2023;84(2)doi:10.4088/JCP.22m14445

187. Rasmusson AM, Marx CE, Jain S, et al. A randomized controlled trial of ganaxolone in posttraumatic stress disorder. *Psychopharmacology*. 2017/08/01 2017;234(15):2245-2257. doi:10.1007/s00213-017-4649-y

188. Kawada T. Effect of ganaxolone in patients with posttraumatic stress disorder. *Psychopharmacology*. 2018/05/01 2018;235(5):1619-1619. doi:10.1007/s00213-018-4892-x

189. Morrow AL, VanDoren MJ, Penland SN, Matthews DB. The role of GABAergic neuroactive steroids in ethanol action, tolerance and dependence. *Brain Res Brain Res Rev*. Nov 2001;37(1-3):98-109. doi:10.1016/s0165-0173(01)00127-8

190. Besheer J, Lindsay TG, O'Buckley TK, Hodge CW, Morrow AL. Pregnenolone and ganaxolone reduce operant ethanol self-administration in alcohol-preferring p rats. *Alcoholism, clinical and experimental research*. 2010;34(12):2044-2052. doi:10.1111/j.1530-0277.2010.01300.x

191. Milivojevic V, Fox HC, Sofuoglu M, Covault J, Sinha R. Effects of progesterone stimulated allopregnanolone on craving and stress response in cocaine dependent men and women. *Psychoneuroendocrinology*. 12/14 2016;65:44-53. doi:10.1016/j.psyneuen.2015.12.008

192. Tannenbaum C, Ellis RP, Eyssel F, Zou J, Schiebinger L. Sex and gender analysis improves science and engineering. *Nature*. 2019/11/01 2019;575(7781):137-146. doi:10.1038/s41586-019-1657-6

193. Health NOoRoWs. NIH Policy on Sex as a Biological Variable. https://orwh.od.nih.gov/sex-gender/nih-policy-sex-biological-variable

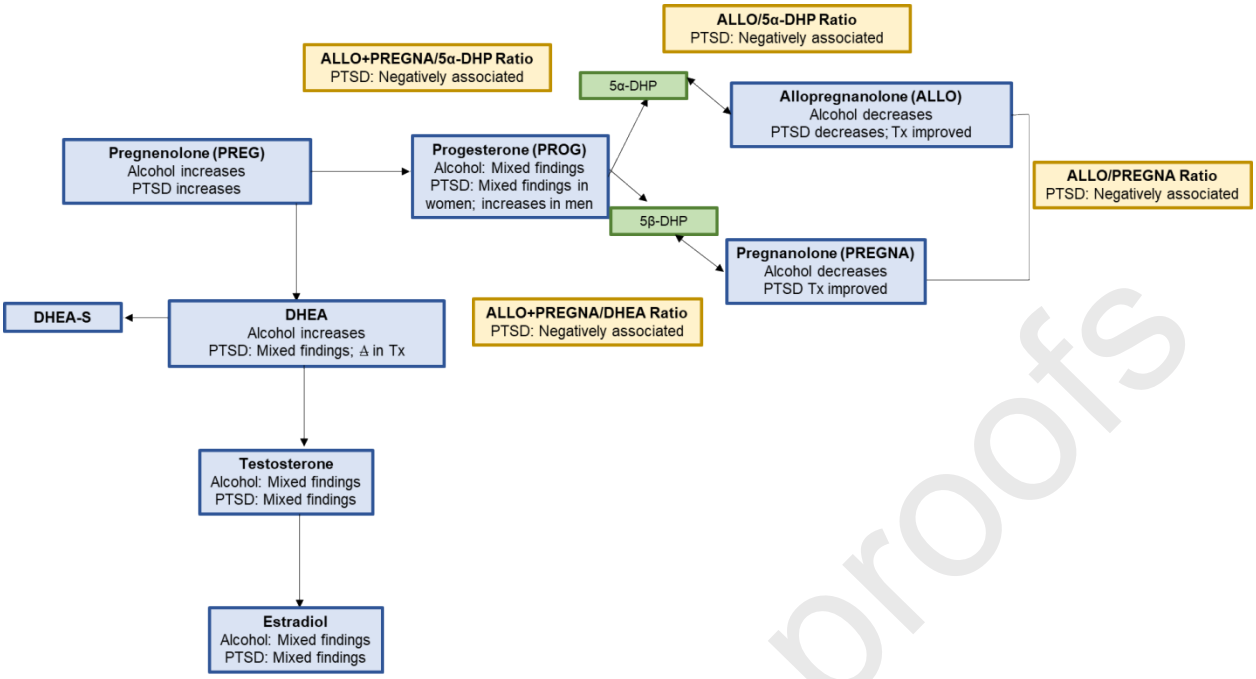
194. Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. *Recent progress in hormone research*. 2002;57:257-276.

195. Chaudhary H, Patel J, Jain NK, Joshi R. The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. *J Ovarian Res*. Sep 26 2021;14(1):125. doi:10.1186/s13048-021-00879-w

Highlights

- Neurosteroids are implicated in the underlying mechanisms of PTSD/AUD and are potential treatments
- Pregnenolone, progesterone & allopregnanolone have the most support for PTSD and AUD
- Few studies have explored the role of neurosteroids for PTSD and AUD concurrently
- There is an inconsistent application of SABV which warrants more rigorous research

Figure 1. Synthesis of neurosteroids with generalized findings.



Note. A simplified depiction of the synthesis of neurosteroids is depicted along with an overall summary of the findings. Findings are related to changes in neurosteroid levels and/or laboratory findings, unless noted as a treatment related finding. Findings including a ratio are depicting a negative correlation between the ratio described and PTSD diagnosis and/or symptoms. In general, there is evidence that allopregnanolone may be advantageous treatment targets for PTSD symptoms and alcohol use, while other neurosteroids demonstrated mixed results. See Table 2 for complete overview of findings.

Table 1. Summary of incorporation of sex as a biological variable into the study design and analyses of neurosteroids and PTSD in past 10 years

	Neurosteroids studied	Included Men and Women	Menstrual Cycle Phase in Study design/analyses	Analyses controlled for SABV	Sex differences planned in analyses	Sex differences reported in results
Kinzel et al., 2020[50]	Preg; ALLO	Yes	No	Yes	No	No
Umminger et al., 2023[52]	Preg; ALLO	Yes	No	Yes	No	No
Marx et al., 2016[51]	Study 1: preg	No	N/A	No	No	No
	Study 2: preg	Yes	No	No	No	No
Cruz et al., 2019[53]	preg; ALLO; pregna	Yes	No	Yes	Yes	Women w/ PTSD ↑ preg &↑pregna vs. women w/out PTSD; Men w/ PTSD ↓ ALLO vs. men w/out PTSD
Nillni et al., 2015[54]	Prog	No	Yes†	No	No	No
Nillni et al., 2020[55]	Prog; E2	No	Yes†	No	No	No
Wegerer et al., 2014[64]	Prog; E2	No	Yes†	No	No	No
Pineles et al., 2018[71]	ALLO	No	Yes†	No	No	No
Pineles et al., 2016a[65]	Prog; E2	No	Yes†	No	No	No
Pineles et al., 2016b[89]	Prog; E2	No	Yes†	No	No	No

Lalonde et al., 2021[56]	Prog; T	Yes	No	Yes	Yes	Women ↓T and↑ Prog than men; ↑E2=lower risk of PTSD
Krinke et al., 2022[57]	Prog, E2; T	No	Yes	No	No	No
Hennessy et al, 2022[58]	Prog; E2	Yes	No	Yes	Yes	Men receiving hydrocortisone: ↑E2= fewer intrusions; ↑Prog=more intrusions Women receiving hydrocortisone: ↑E2= more intrusions
Inslicht et al., 2014[59]	Prog; ALLO	Yes	No	Yes	Yes	↑Prog in women with PTSD vs. controls as compared to men ↑ALLO in women vs. men
Juster et al., 2016[60]	Prog; E2	Yes	No	Yes	Yes	Men: Prog negatively correlated with reactive cortisol Women: E2 and T negatively correlated with reactive cortisol
Stephens et al., 2016[62]	Prog; E2; T	Yes	Yes†	Yes	Yes	Men: T negatively correlated with cortisol Women: Prog negatively correlated to ACTH and cortisol
Pineles et al., 2020[72]	ALLO; Pregna; DHEA	No	Yes†	No	No	No
Kim et al., 2020[63]	Pregna; ALLO	No	N/A	No	No	No
Rauch et al., 2020[68]	Pregna; ALLO	Yes	No	No	Yes	Male responders: ↑ALLO levels and ↑baseline Pregna

						levels
Rasmusson et al., 2019[70]	Prog; ALLO; Pregna; DHEA; DHEA-S	No	N/A	No	No	No
Reijnen et al., 2015[74]	T	No	N/A	No	No	No
Farina et al., 2017[77]	T	No	N/A	No	No	No
Lehrner et al., 2016[76]	DHEA	No	N/A	No	No	No
Feklicheva et al., 2022[75]	T	No	N/A	No	No	No
Deuter et al., 2021[80]	T	No	Yes	No	No	No
Karlovic et al., 2012[81]	T	No	N/A	No	No	No
Josephs et al., 2017[78]	T	Yes	No	Yes	No	No
Cobb et al., 2018[79]	T	Yes	No	Yes	No	No
Rieder et al., 2022a[82]	E2	No	Yes[α]	No	No	No
Hack et al., 2022[83]	E2; Prog	No	No	No	No	No
Rieder et al., 2022b[85]	E2	No	Yes[α]	No	No	No
Miedl et al., 2018[94]	E2	No	Yes	No	No	No
Sartin-Tarm	E2	No	No	No	No	No

et al., 2020 [84]						
Glover et al., 2012[86]	E2	No	No	No	No	No
Glover et al., 2013[87]	E2	No	Yes	No	No	No
Antov et al., 2014[99]	E2	Yes	Yes	Yes	Yes	Women in early follicular phase ↑ fear response at fear extinction vs. controls; women in midcycle phase ↓ fear response vs. controls
Soni et al., 2013[90]	E2	No	Yes	No	No	No
Franke et al., 2022[91]	E2	No	Yes	No	No	No
Ferree et al., 2012[96]	E2; Progestin	No	No	No	No	No
Bartholomew et al., 2022[97]	E2	Yes	Yes	Yes	Yes	Women on hormonal contraceptives (HCS) ↑ change in skin conduction response during fear conditioning vs. men and women in early follicular phase; Women on HCs ↓change in SCR during extinction then men.
Graham et al., 2013[98]	E2	No	Yes	No	No	No
Jergovic et al., 2015[101]	DHEA	No	N/A	No	No	No
Orta et al., 2020[102]	DHEA	No	N/A	No	No	No
Mouthaan et al., 2014[103]	DHEA-S	Yes	No	Yes	No	No

Usta et al., 2016[104]	DHEA-S	No	No	No	No	No
Ralph et al., 2017[105]	DHEA	Yes	No	No	No	No
Bicanic et al., 2013[109]	DHEA-S	No	No	No	No	No
Golier et al., 2014[110]	DHEA-S	No	N/A	No	No	No
Usta et al., 2018[111]	DHEA-S	Yes	No	Yes	No	No
Nijdam et al., 2015[112]	DHEA-S	Yes	No	Yes	No	No
Kim et al., 2013[113]	DHEA-S	Yes	No	Yes	No	No
Van Voorhees et al., 2013[107]	DHEA	Yes	No	Yes	No	No
Blasco-Ros et al., 2014[108]	DHEA	No	No	No	No	No
Van Voorhees et al., 2014[106]	DHEA; DHEA-S	Yes	No	Yes	No	No

Note. SABV= sex as a biological variable; Preg=pregnenolone; ALLO=allopregnanolone; Pregna=pregnanolone; Prog=progesterone; E2=estradiol; DHEA= dehydroepiandrosterone; T=testosterone; N/A= not applicable; ↑= increased or greater; ↓= decreased or less than; †=menstrual cycle subphase confirmed by blood analysis; α=menstrual cycle subphase confirmed by salivary assessment

Table 2. Generalized summary of findings

	Preg			Prog			ALLO			Pregna			T			E2			DHEA/DHEA-S		
	All	W	M	All	W	M	All	W	M	All	W	M	All	W	M	All	W	M	All	W	M
Alcohol																					
AUD	↑	↑	NE	NE	NE	↑/↓	↑/↓	↑/↓	↑/↓	NE	NE	↓	↑/↓	⩲	↑/↓	NE	NE	NE	↑	↑/↓	↑
Consumption	↑	⩲	↑	NE	↑/↓	NE	↓	↓	↓	NE	NE	NE	NE	↑/↓	↑/↓	NE	↑	↓	↓	↑	↑
Craving	NE	NE	NE	NE	↑/↓	NE	NE	NE	↑	NE	NE	NE	NE	NE	↑	NE	NE	NE	NE	NE	NE
Positive subjective ratings	NE	⩲	↑	NE	NE	NE	NE	NE	↑	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
PTSD																					
Diagnosis	NE	↑	⩲	↑/↓	⩲	↑	NE	↓	↓	NE	NE	NE	NE	NE	↑/↓	NE	↓	↑/↓	↑/↓	↑/↓	↑/↓
Fear Conditioning	NE	NE	NE	NE	⩲	NE	NE	↑	NE	NE	NE	NE	NE	NE	NE	NE	↑/↓	↓	NE	↑	NE
PTSD Symptoms	NE	NE	↑/↓	NE	↑/↓	↑	↓	↓	↓	NE	NE	↓	↑	NE	↓	NE	↑/↓	NE	↓	↑/↓	⩲
Treatment	NE	NE	NE	NE	NE	NE	NE	NE	↑	↑	NE	↑	NE	NE	NE	NE	NE	NE	⩲	⩲	⩲

respons e							

Note. W=women; M=men; Preg=Pregnenolone; Prog= progesterone; ALLO= allopregnanolone; Pregna= Pregnanolone; T=Testosterone; E2=Estradiol; DHEA= Dehydroepiandrosterone; DHEA-S= Dehydroepiandrosterone-Sulfate; ↑= increased hormone levels; ↓= decreased hormone levels; ↑/↓= mixed evidence; NE= no available evidence; ≙=no differences observed.

Table 3. Summary of incorporation of sex as a biological variable into the study design and analyses of neurosteroids and Alcohol in past 10 years

	Neurosteroids studied	Included Men and Women	Menstrual Cycle Phase in Study design/analyses	Analyses controlled for SABV	Sex differences planned in analyses	Sex differences reported in results
Karkkainen et al., 2016[115]	Preg; DHEA; T	Yes	No	Yes	Yes	Men only analyses showed almost identical findings
Gatta et al., 2021[125]	Prena; ALLO	No	No	No	No	No
Li et al., 2013[147]	E2; T	No	Yes	No	No	No
Hirko et al., 2014[131]	E2; T; Prog; DHEA; DHEA-S	No	Yes†	No	No	No
Lanet Group, 2013[146]	E2; T; DHEA-S	No	Yes†	No	No	No
Vatsalya et al., 2012[137]	T; E2	Yes	Yes	Yes	Yes	Men: ↓T Women: ↑E2
Tin Tin et al., 2021[140]	T; E2	No	Yes†	No	No	No
Schliep et al., 2015[133]	T; E2; Prog	No	Yes	No	No	No
Braams et al., 2016[145]	T	Yes	Yesα	No	No	No
De water et al., 2013[139]	T; E2	Yes	No	Yes	Yes	↑T and E2 in boys is linked to onset of alcohol use; ↑E2 related to ↑alcohol quantity in boys

Peters et al., 2015[143]	T	Yes	Yes[α]	Yes	Yes	↑T in boys; no findings in girls
Heberlein et al., 2016[142]	T	No	N/A	No	No	No
Jensen et al., 2014[138]	T	No	N/A	No	No	No
Holzhauer et al., 2020[117]	Prog	No	Yes[α]	No	No	No
Martel et al., 2017[116]	Prog; E2	No	Yes[α]	No	No	No
Joyce et al., 2018[118]	Prog; E2	No	Yes	No	No	No
Weinland et al., 2021[119]	Prog	Yes	Yes	No	No	Postmenopausal Women= ↑Prog is related to ↓ craving; Men with AUD = ↑Prog, that decreased over time
Hayaki et al., 2020[120]	Prog; E2	No	Yes	No	No	No
Barone et al., 2023[187]	Prog; E2	No	Yes	No	No	No
Milivojevic et al., 2016[183]	ALLO; T	Yes	No	Yes	Yes	Men/Women equal levels of ALLO when separated in High/Low ALLO groups
Peltier et al., 2020[148]	T; E2	No	No	No	No	No
Hasirci et al., 2017[124]	ALLO	Yes	No	Yes	Yes	↑ ALLO in VTA in men/women; ↑ ALLO in SMN in AUD males vs. controls

Hartman et al., 2016[132]	E2	No	Yes[β]	No	No	No
Playdon et al., 2018[134]	E2	No	N/A	No	No	No
Warren et al., 2021[135]	E2; Prog	Yes	Yes	No	No	No
Wemm et al., 2013[151]	DHEA	No	No	No	No	No
Nagaya et al., 2012[149]	DHEA-S	No	N/A	No	No	No
Aluja et al., 2023[150]	DHEA-S	No	N/A	No	No	No
Weinland et al., 2022[153]	DHEA-S	Yes	No	Yes	Yes	↓ DHEA-S in men and women in AUD treatment Women: ↓ DHEA-S in AUD vs. controls
Schrieks et al., 2016[152]	DHEA	No	N/A	No	No	No
Ransome et al., 2017[154]	DHEA-S	Yes	No	Yes	No	No
Obasi et al., 2017[155]	DHEA	Yes	No	Yes	No	No

Note. SABV= sex as a biological variable; Preg=pregnenolone; ALLO= allopregnanolone; Pregna=pregnanolone; Prog=progesterone; E2=estradiol; DHEA= dehydroepiandrosterone; T=testosterone; N/A= not applicable; ↑= increased or greater; ↓= decreased or less than; †=menstrual cycle subphase confirmed by plasma analysis; α=menstrual cycle subphase confirmed by salivary assessment; β= menstrual cycle subphase confirmed by urinary assessment; VTA=ventral tegmental area; SNM substantia nigra pars medialis.