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The GABA system, a new target for medications against cognitive impairment—Associated with neuroactive steroids

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The prevalence of cognitive dysfunction, dementia, and neurodegenerative disorders such as Alzheimer's disease (AD) is increasing in parallel with an aging population. Distinct types of chronic stress are thought to be instrumental in the development of cognitive impairment in central nervous system (CNS) disorders where cognitive impairment is a major unmet medical need. Increased GABAergic tone is a mediator of stress effects but is also a result of other factors in CNS disorders. Positive GABA-A receptor modulating stress and sex steroids (steroid-PAMs) such as allopregnanolone (ALLO) and medroxyprogesterone acetate can provoke impaired cognition. As such, ALLO impairs memory and learning in both animals and humans. In transgenic AD animal studies, continuous exposure to ALLO at physiological levels impairs cognition and increases degenerative AD pathology, whereas intermittent ALLO injections enhance cognition, indicating pleiotropic functions of ALLO. We have shown that GABA-A receptor modulating steroid antagonists (GAMSAs) can block the acute negative cognitive impairment of ALLO on memory in animal studies and in patients with cognitive impairment due to hepatic encephalopathy. Here we describe disorders affected by steroid-PAMs and opportunities to treat these adverse effects of steroid-PAMs with novel GAMSAs.

Keywords: allopregnanolone, dementia, GABA-A receptor, memory, neurosteroids

Abbreviations: [Cl^-], chloride ion concentration; μM, nM, micro molar, nano molar; 3α5β-P, 3α-OH,5β-pregnanolone; 3α5β-THDOC, tetrahydrodeoxycorticosterone; AD, Alzheimer's disease; ALLO, allopregnanolone; APP, amyloid precursor protein/amyloid-beta precursor protein; GABA, gamma amino butyric acid; GAMSA, GABA-A receptor modulating steroid antagonists; GR3027, golexanolone an oral GAMSA; HE, hepatic encephalopathy; HRT, hormone replacement therapy; Iso-allo, 3β-hydroxy-5α-pregnan-20-one; MPA, medroxyprogesterone acetate; MWM, Morris water maze; PAM, positive allosteric modulator of GABA-A receptor; PS, pregnenolone sulfate; PTSD, post-traumatic stress disorder; TG-APPSwe/Arc/PS1 mice, transgenic mice with Swedish, Artic, PS1 mutations in APP; TSPO, translocator protein

Introduction

The population of the industrialized countries is increasing with age. At the same time, the prevalence of memory, learning disorders, and dementia is increasing [1]. Distinct types of chronic stress are believed to be instrumental in the development of cognitive impairment. This review will look at the effects of stress on cognitive processes as well as the involvement of neurosteroids and GABA-A receptors in that process.

Stress, memory, and steroid-PAM in acute stress

Acute stress can in certain situations both increase and decrease the strength of memories, but in

chronic stress, the ability to remember decreases [2]. Acute stress disorder and post-traumatic stress disorder (PTSD) can occur in severe acute stress with large activation of the adrenal cortex, which often leads to memory impairment [3, 4]. In acute stress, there is a pronounced activation of the adrenal gland's production of steroids. The increase in cortisol production in humans and corticosterone in rodents is well known and described in acute stress, whereas the parallel production of positive GABA-A receptor modulators (steroid-PAMs) has been less described. One of the most potent steroid-PAMs is allopregnanolone (ALLO), a metabolite of progesterone that is formed in the adrenal cortex. ALLO production increases during stress at the same time as other stress steroids, such as cortisol in humans and corticosterone in rodents [5–8]. Increases in steroid-PAM correlate with changes in amygdala volume and anxiety symptoms in women [9, 10]. Many repeated acute stress events can eventually turn into chronic stress and thus damage cognitive functions [11]. However, even at acute stress under chronic stress conditions, steroid-PAM production, the acute stress steroid levels, increase in central nervous system (CNS) [5, 12, 13].

Memory and cognitive function in chronic stress

There are several types of chronic stress, like PTSD, psychosocial work stress, burnout syndrome, and/or chronic fatigue disorder often have cognitive problems [3, 11, 14–16]. Rats with chronic stress show downregulated ALLO production, and the plasma concentration is low. Female and male rats under chronic stress show a larger acute stress response with higher ALLO concentrations than during non-chronic stress conditions when exposed to an acute stressor [6]. ALLO acute stress response in humans under chronic stress conditions is comparable to that seen in rats, but in women, the response is smaller compared to men [17]. In burnout syndrome, memory is often disturbed. With repeated stress—work-related, psychosocial, and/or emotional of various kinds—the risk of dementia increases [18–20]. Stress in animal models of Alzheimer's disease (AD) impairs memory and learning [21, 22]. This means that chronic stress must be considered a risk for developing dementia and AD [23]. The biological relationship between memory disorders and chronic stress is not known, but steroid-PAM, elevated chronic GABAergic tone, and change in steroid-PAM sensitivity in the GABA-A system may be factors of interest to investigate further.

Steroid-PAM in chronic stress and disorders with cognitive impairment

In the case of chronic stress, steroid production decreases to low concentrations in the blood, so also for ALLO [24]. However, this does not occur in all individuals, and some continue with high steroid production even under chronic stress conditions. In these individuals, poorer cognitive function is related to increased cortisol concentrations in the blood [14, 25]. In addition, GABA-A receptor sensitivity to steroid-PAM appears to increase due to the altered composition of GABA-A receptor subunits and thus with different sensitivity to steroid-PAM [26]. GABA-A receptor sensitivity to ALLO and other PAMs can be determined using GABA-A receptor-controlled functions—for example, saccadic eye velocity (SEV) and sedation [27, 28]. In this regard, women with PTSD showed reduced sensitivity to a positive allosteric modulator, namely, diazepam (a benzodiazepine) both in terms of sedation and SEV [29]. The PTSD group showed no difference in the early initial ALLO response compared with controls but showed a faster recovery in SEV after ALLO injection. This suggests a GABA-A receptor subtype change in the PTSD group to a receptor subtype unresponsive to benzodiazepines—namely, $\alpha 4,\beta x,\delta$, the subunit in the region responsible for the SEV effect. In patients with burnout syndrome, a similar response is noted with a reduced sensitivity to benzodiazepines but an increased effect of ALLO. We know that the $\alpha 4,\beta x,\delta$ subunit is highly sensitive to ALLO but insensitive to benzodiazepines, which would indicate an upregulation of the $\alpha 4,\beta x,\delta$ subunit GABA-A receptor in these disorders [30].

GABA-A receptors involved in memory

In adults, an activation of the GABA-A receptor leads to an inward flow of chloride ions. This leads to a hyperpolarization—that is, inhibition of the neuron [31]. In fetuses, in neuronal stem cells and certain neurological disorders, the chloride flow is reversed when the GABA-A receptor opens, and GABA thus becomes excitatory [32–34]. Postsynaptic GABA-A receptors can be localized to the synaptic cleft (intra-synaptic) but also outside the synaptic cleft itself and are then called extra-synaptic receptors [35]. Intra-synaptic GABA-A receptors generally contain $\alpha 1$, $\alpha 2$, or $\alpha 3$ subtypes. These receptors are less sensitive to GABA and require

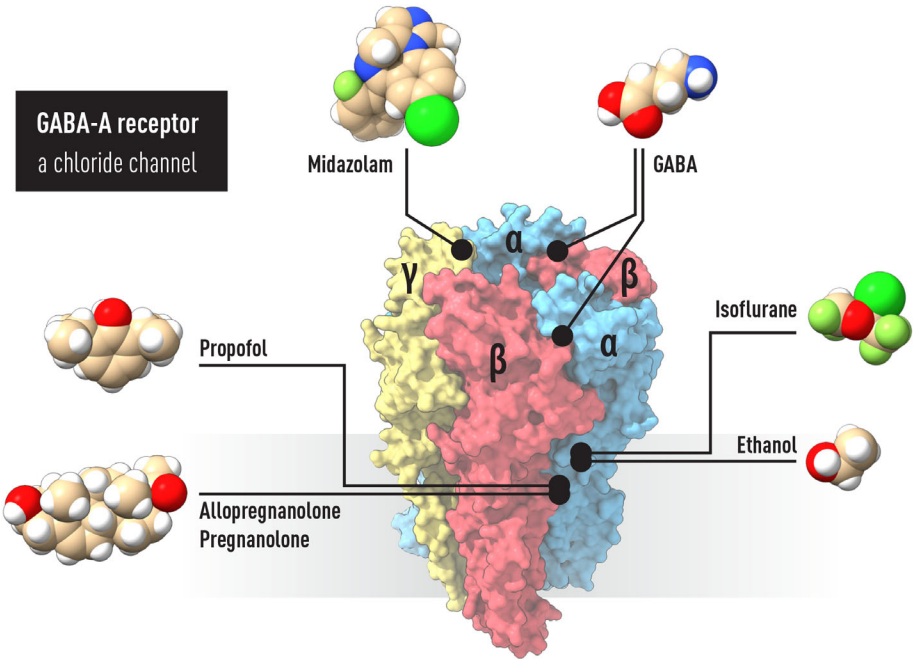

Fig. 1 *The GABA-A receptor is a chloride ion channel and target for among other compounds GABA, positive allosteric modulators, for example, neurosteroids, barbiturates, benzodiazepines, ethanol, anesthetic compounds. The GABA-A receptor has 5 subunits, α units 1–6, β units 1–3, γ units 1–3, δ, ε, and θ units. Approximately 20 different subunit combinations exist in the brain located to specific brain areas and related to specific functions. Receptors with α units 1–3 are mainly intra-synaptic and need high GABA concentration for opening, whereas α units 4–6 are extra synaptic and need lower concentrations of GABA and stand open for longer periods and increases the GABAergic tonus. For more detailed information care for Refs. [39,26,35].*

high GABA concentrations to open (typical physiological concentrations 100 μM), whereas the extrasynaptic receptors generally contain α4, α5, or α6 and require lower GABA levels for activation (typical physiological concentrations 0.3 μM) [26, 36, 37]. The GABA-A receptor has about 20 different subtype sets in the brain, and these sets have specific localization in different areas of the brain (Fig. 1). Thus, certain subtypes will be able to influence the function of the brain area where the receptor is located [38]. More information about receptor subtype area function is described elsewhere [31, 39]. The receptor type with the α5 subunits is well known to be related to cognition and is found in the hippocampus. Subtypes containing α4,βx,δ are abundant in the thalamus and are highly sensitive to steroid-PAM, which also controls their expression [26, 40].

Acute and chronic treatment with positive GABA-A receptor modulators on memory

Many drugs affect GABA-A receptors—for example, benzodiazepines [41, 42], barbiturates [43], ethanol [44], zinc [45], and steroid-PAMs [13, 46–49]. Several steroid-PAMs—such as ALLO, 3α-OH,5β-pregnanolone (3α5β-P), tetrahydrodeoxycorticosterone (3α5β-THDOC), and 3α-OH,5α-androstanediol—have CNS depressant effects that are even able to induce anesthesia. Such agents are all positive GABA-A receptor-modulating steroids—that is, steroid-PAMs [50–52]. Several steroid-PAMs can impair memory and learning at low concentrations. For example, ALLO reduces hippocampal neuronal activity in rats [53, 54]. Rats given ALLOs have impaired learning and poor memory when evaluated in the Morris water maze (MWM) memory model [55, 56]. In women, episodic memory is blocked after an ALLO injection [57], and that is interesting because episodic memory is disrupted early in AD [58]. Long-term potentiation is a phenomenon linked to memory development in the hippocampus; steroid-PAM inhibits that development [59], and ALLO inhibits the cholinergic effect in the hippocampus [60]. In humans, we have seen that progesterone and/or its metabolite ALLO impair the ability to retrieve memories

from distinct parts of the brain [61]. Positive GABA-A receptor modulators, benzodiazepines, barbiturates, and ethanol cause in humans long-term acute and permanent cognitive impairments [62–64] and rats [65]. Positive GABA-A receptor modulators inhibit neurotransmission and thus cognition by activating GABAergic mechanisms [66, 67]. This suggests that exposure to positive modulation of GABA-A receptors during prolonged periods increases the risk of cognitive impairment.

Medroxyprogesterone MPA treatment and dementia development

Hormone replacement therapy (HRT) in postmenopausal women with the steroids estrogen and progestin has for a long time been discussed for memory improvement and protection against dementia [68, 69]. HRT with estrogen plus medroxyprogesterone acetate (MPA) is a common variant of HRT. In a large study started to investigate whether HRT could improve memory function and prevent the development of dementia—the Women's Health Initiative Memory Study—postmenopausal women were treated with estrogen plus MPA for 4–7 years in a placebo-controlled randomized trial. The results of the study were contrary to expectations in that the number of women with suspected dementia was twice as many in the estrogen plus MPA group compared to the placebo group [70]. In the group treated with estrogen alone, there was no difference compared to the placebo group [71]. The increase in suspected dementia was also not dependent on ischemic lesions or stroke, and increased dementia rate was due to a biological factor that was likely to be MPA [72, 73]. MPA can also prevent and attenuate the positive effects of estrogen [74]. Studies in animal models have shown impaired ability to learn and remember with estradiol plus MPA treatment, or with MPA treatment alone compared to estrogen treatment [75–77]. MPA has the same properties as progesterone and ALLO—that is, it causes sedation and anesthesia [51, 78, 79]. This suggests that MPA acts via the GABA-A receptor. With the results of MPA treatments described above, especially on memory and learning, it was of interest to investigate whether MPA has a modulatory effect on GABA-A receptors.

MPA is a positive GABA-A modulator (PAM)

There is a lack of knowledge about MPA effects on subtypes of GABA-A receptors involved in cognition and mood. We have therefore performed studies of MPA on recombinant human GABA-A receptors [80]. Our studies in human embryonic kidney 293 cell lines permanently expressing the $\alpha1\beta2\gamma2$L, $\alpha5\beta3\gamma2$L, or $\alpha2\beta3\gamma2$S subtypes—using electrophysiological patch-clamp technology—examined MPA compared to well-known steroid-PAMs such as THDOC and ALLO. We chose the $\alpha1$ subtype because it is the most common in the CNS and has been the receptor subtype most often investigated in previous studies [35, 81, 82]. The $\alpha5$ subtype is found in the hippocampus and is related to memory, learning, and dementia development [31, 83, 84]. Depression is a common side effect of MPA, and the $\alpha2$ subtype has been discussed in association with depressive symptoms [39, 85]. The results show that MPA has large and differentiated effects as a positive modulator of GABA's effect and has a direct activating effect on $\alpha5\beta3\gamma2$L and $\alpha2\beta3\gamma2$S GABA-A receptors. However, MPA did not affect the $\alpha1\beta2\gamma2$L GABA-A receptor evaluated up to a concentration of 10 μM. Patch-clamp studies were also performed in cells from the preoptic area of the rat hypothalamus. MPA also showed its direct effect without adding GABA in rat hypothalamic cells. These results demonstrate that MPA is a positive allosteric modulator of GABA-A receptor subtypes $\alpha5\beta3\gamma2$L and $\alpha2\beta3\gamma2$S, and influences cells taken from the hypothalamic preoptic area [80].

Effect of PAM on AD TG mice

As mentioned above, progesterone has anticonvulsant and anesthetic effects [79, 86]. These effects are due to the metabolite ALLO, which is a positive GABA-A receptor modulator more potent than MPA [87]. The GABA-A receptor is in adult mature neurons the major inhibitory receptor, and ALLO can induce anesthesia via its action on it [48, 51]. In the rat hypothalamus, a dosage of only 2 nM is needed to activate the GABA-A receptor [88]. This concentration is reached in the CNS during acute stress, for example, and in women during the menstrual cycle [5, 12]. Hippocampus is a CNS region intricately linked to cognition [89, 90]. Changes in the hippocampus are seen early in AD development, resulting in a poorer ability to consolidate new experiences (episodic memory). Disorientation and reduced spatial perception are also early symptoms of AD. A frequently used memory-related technique in animal experiments is the MWM because it is particularly suitable for investigating spatial hippocampus-related memory [91]. ALLO concentrations as in mild stress continuously elevated or given as repeated injections of ALLO several times a week lead to reduced mem-

ory and poorer learning ability as well as the faster progression of AD disease in transgenic (TG) AD mice [92–94]. In our studies, a low-stress concentration of subcutaneous ALLO was given continuously with Alzet mini pumps systemically for 1 month to TG-APPSwe/Arc mice, and for 3 months to TG-APPSwe/PS1 mice (Fig. 2). Both mouse types have double mutations in the amyloid-beta precursor protein (APP) gene (Swedish/Artic or Swedish/PSEN1δE9), giving an increase in Aβ-protein production and plaque formation. In both experiments, the TG mice had impaired learning and memory compared to placebo-treated mice and wild-type mice [92, 93]. The experiments were done 1 month after the Alzet pumps, and thus, the ALLO exposure was removed. At 1 month after the removal of the Alzet pumps, no external ALLO remained in the body at the time of the memory and learning test. This indicates that the memory and learning difficulties were not due to the presence of the drug in the brain, but rather that the ALLO treatment had caused permanent damage to memory and learning ability. The results show that chronically mildly elevated levels of ALLO permanently impair memory. One month after the medication had stopped, and the memory impairment was still present in the MWM test (Fig. 2). ALLO had a greater effect on male APPSwe/PS1 mice compared to females. Why there was a gender difference is not known. TG-APPSwe/Arc mice showed impaired learning already after 1 month of ALLO treatment in low-stress concentration. In the TG-APPSwe/Arc mice, the effect was as great in females as in males, especially in the so-called probe test where the mice must find a hidden platform under the water surface in the pool. Comparable results have been obtained with frequent injections of ALLO (3 injections/week) to 3xTgAD mice, where impairment of memory and learning was shown. However, it has been shown that ALLO injections at longer intervals—that is, weekly injections in AD mice with a pharmacological dose of ALLO—induce growth in neural stem cells that form new neurons in the hippocampus [94, 95]. It is well known that immature progenitor cells are excited by GABA and ALLO and not inhibited as in mature neurons. We also treated wild-type mice with ALLO for 5 months. The wild-type mice also had a residual memory impairment and a smaller hippocampal volume [96]. Previously, however, we have shown that ALLO has a tolerance development toward high concentrations, but despite this, we saw memory impairment in the wild-type mice [96, 97]. However, the concentration obtained continuously in the wild-type mice was lower than in the rat MWM experiments, and the development of tolerance is dependent on dosage and frequency of administration [96, 97]. A summary is given in Table 1.

Human diseases with high steroid-PAM concentrations, and impaired memory and learning

A disease with high ALLO levels in the CNS and blood is hepatic encephalopathy (HE). High GABAergic tone has also been shown to be associated with impaired memory and learning [98–101]. CNS concentrations of ALLO have been investigated in autopsy material, which are increased compared to patients without HE [102]. In animal models of HE, levels of ALLO and THDOC are also high [98, 103]. The most important pathological factor in HE is an increase in ammonia production. Ammonia stimulates the production of steroid-PAM by increasing the transport of cholesterol into mitochondria through the upregulation of translocator protein (TSPO) [104]. TSPO is also a marker of neuroinflammation that is increased in HE [105–107]. ALLO formation increases due to the increase in the intramitochondrial production of pregnenolone and by the cytoplasmic enzymes further down the pathway to progesterone and ALLO [104, 108, 109]. Primary biliary cholangitis is another liver disease with high ALLO concentrations and cognitive impairment [110]. Obesity and overweight are known to have high ALLO levels, even though cognitive impairment is not a prominent symptom [111]. Menstrual cycle-related mood changes are known to develop in parallel with increasing ALLO levels during the luteal phase of the menstrual cycle [112]. Conditions such as burnout syndrome, PTSD, and premenstrual dysphoric disorder show a different GABA-A receptor sensitivity to ALLO compared to controls [27, 29, 30].

GABA-A modulating steroid antagonists (GAMSAs)

We know that ALLO enhances the effect of GABA, and that ALLO binds to its own sites on the GABA-A receptor [113]. A minor change in ALLO's chemical structure at the hydroxy group in the three positions with the hydroxy group in the 3β position instead of the 3α position makes a marked difference in the effect on the GABA-A receptor. ALLO's 3β-isomer (3β-hydroxy-5α-pregnan-20-one [iso-allo]) has no intrinsic effect on GABA-A receptors [114]. On the other hand, we have shown that iso-allo can counteract

Fig. 2 *Three months continuous allopregnanolone treatment (low-stress concentrations) induces permanent memory deficit in Tg-AD mice ($APP_{Swe/PS1}$). Top picture shows design of the study. First habituation 9 weeks, treatment started at 10 weeks of age, and stopped at 22 weeks of age. Then a 4 weeks rest before memory/learning testing. One month after end of treatment Morris water maze learning test was performed. Male results in Morris water maze test are shown below to the left. Data is shown as mean ± SEM. *$p < 0.05$ Swe/PS1, allopregnanolone versus Swe/PS1, vehicle on Day 4–6. **$p < 0.01$ Swe/PS1, allopregnanolone versus wild-type, allopregnanolone on Day 1–6. To the right are shown the percentage mice remembering/learned (gray) or not learning (black) to find the hidden platform, sexes combined. (A) ***$p < 0.001$ Swe/PS1 allopregnanolone versus wild-type allopregnanolone, (B) **$p < 0.001$ Swe/PS1 allopregnanolone versus Swe/PS1 vehicle. Comparable results are obtained in 3xTg-AD mice given frequent allopregnanolone injections [94]. AD, Alzheimer's disease. Source: "Reprinted from Ref. [92], with permission from IOS Press."*

Table 1. *Effect of steroid positive allosteric modulators (PAM) and GABA-A modulating steroid antagonists (GAMSA).*

Type of compound	Effect	Reference
Steroid-PAM	Positive allosteric modulators (PAM) of GABA-A receptors	[13],[48].
Steroid-PAM	Anesthetic	[51],[135].
Steroid-PAM	Sedative and decrease saccadic eye velocity	[133].
Steroid-PAM	Anticonvulsive, antiepileptic effects	[53],[86].
Steroid-PAM	ALLO reduces postpartum depression	[136].
Steroid-PAM	Reduces hippocampal neuronal activity in rats	[54].
Steroid-PAM	Impair memory and learning in rodents	[55],[53],[119].
Steroid-PAM	Intermittent ALLO increases hippocampal progenitor cell proliferation and improve memory	[94],[137].
Steroid-PAM	Continuous stress levels of ALLO give reduced memory and learning and faster AD progression in transgenic AD mice	[92–94].
Steroid-PAM	Chronically mildly elevated ALLO level permanently impair memory in wild-type mice	[96].
Steroid-PAM	Block episodic memory in humans	[97].
Steroid-PAM	In humans impairs retrieval of memories, fMRI	[61].
Steroid-PAM	Medroxyprogesterone acetate (MPA) is a PAM of GABA-A receptor subtypes $\alpha5\beta3\gamma2$L and $\alpha2\beta3\gamma2$S but not $\alpha1\beta2\gamma2$L	[80].
Steroid-PAM	MPA in rodents impair learning and memory	[75–77].
Steroid-PAM	MPA treatment for 4–7 years doubles suspected dementia vs. placebo. Women's Health Initiative Memory Study	[70].
GAMSA	ALLO's 3β-isomer, (3β-hydroxy-5α-pregnan-20-one, iso-allo) has alone no effect on GABA-A receptors	[114],[138].
GAMSA	Iso-allo antagonizes ALLO/THDOC's effects, e.g., in hippocampal slices	[116].
GAMSA	Iso-allo inhibited ALLO-induced Cl^- uptake in rat cortex homogenates	[117].
GAMSA	Iso-allo and 3β-steroids reduces PAM effect in rat hypothalamic neurons, patch-clamp studies	[13].
GAMSA	Iso-allo antagonizes anesthetic effect of ALLO in rats	[115].
GAMSA	UC1011 antagonized ALLOs impairment of learning and memory in rat Morris Water Maize	118
GAMSA	GR3027 inhibits the effect of PAMs such as ALLO and THDOC	[28].
GAMSA	GR3027 improves motor skills, coordination memory learning in rat models of hepatic encephalopathy (HE)	[28].
GAMSA	GR3027 reduces signs of systemic inflammation	[106].
GAMSA	GR3027 lowers TNFα and IL-10 levels and deactivates activated microglia and astrocytes in rats	[106].
GAMSA	GR3027 antagonizes ALLO induced sedation and slowing of saccadic eye velocity in humans	[122].
GAMSA	GR3027 shows in HE patients' effect on an objective electroencephalogram (EEG) measure and on memory, sleeping score, and reaction time	[134].

Abbreviations: AD, Alzheimer's disease; ALLO, allopregnanolone.

ALLO/THDOC's effects—for example, in hippocampal slices, concentration-dependent. In the experiment, we obtained a complete block of the ALLO effect with the same concentration of iso-allo as ALLO [115, 116]. Using a different technique, we studied the GABA-mediated flux of chloride ions [Cl^-] through the GABA-A receptor. In cortex homogenates from adult rats, iso-allo markedly inhibited ALLO-induced Cl-uptake. This result indicates that iso-allo or a similar substance

could be used as a drug against diseases caused by steroid-PAMs [117]. We obtained comparable results when we studied 3β-steroids in individual rat hypothalamic neurons using the patch-clamp technique—that is, ALLO's effect was reduced by the 3β-steroids [13]. The disadvantage of iso-allo is that it must be given parenterally. However, we have developed a new orally active 3β-steroid—GR3027 (GAMSA)—that can selectively antagonize ALLO- and THDOC-enhanced activation of GABA-A receptors. A summary is given in Table 1.

Novel GABA-A-modulating steroid antagonists (GAMSA) effects on memory

To investigate whether it is possible to counteract ALLO's negative effect on memory, we gave rats a combination of ALLO plus a GAMSA (3β-20β-dihydroxy-5α-pregnane) and compared the combination against the same dose of ALLO alone. GAMSA (3β-20β-dihydroxy-5α-pregnane) significantly shortened the time until the rats found the platform during a 5-day test period compared to the ALLO-injected group, and memory performance was on par with the placebo-treated group [118]. Pregnenolone sulfate (PS) is a GABA antagonist, and it also has antagonistic effects against ALLO [119]. However, PS works differently than 3β-hydroxysteroids because it is a GABA antagonist [117, 120] and can induce epileptogenic seizures like, for example, bicuculline [121]. We have developed an oral 3β-OH compound GR3027 because oral preparation is easier to administer as a drug than a parenteral preparation [122]. With GR3027, we can inhibit the effect of positive GABA-A receptor modulators such as ALLO and THDOC. We have identified HE as a group for which treatment with GR3027 against cognitive and motor impairment could be appropriate. We have investigated GR3027 in two rodent models of HE. The rat models both have increased ammonia production as a basis. One model induced hyperammonemia by having ammonia in the food. In the second model, a portacaval shunt was surgically performed. In both models, the rats had impaired motor skills and impaired memory. GR3027 significantly counteracted the HE symptoms in motor coordination, memory, and learning [122].

GAMSA in relation to neuroinflammation

In the CNS, microglia and astrocytes respond to various brain insults through activation. This activation occurs with neuroinflammation—for example, with hyperammonemia. An enhancement of the GABAergic effect occurred with the activation of microglia and astrocytes. The intracellular [Cl^-] concentration in the neuron decreases through an increase in the effect of the chloride pump (KCC2) out of the cell [123–125]. TSPO increases during neuroinflammation, which increases cholesterol intake to the mitochondria and thus pregnenolone synthesis. Via cytoplasmic enzymes, ALLO is formed, which leads to an increase in the concentration of ALLO in the brain. We know that ALLO is elevated in the CNS of rats with hyperammonemia [126, 127] and in autopsy material from brains of patients with cirrhosis [102]. Studies in rats with hyperammonemia show that treatment with GR3027 reduces systemic inflammation [106]. GR3027 lowers, for example, the TNFα and IL-10 levels, and turns off the activation of microglia and astrocytes in the hippocampus and cerebellum. At the same time, cognition and motor skills are improved, and coordination is normalized. However, the mechanisms behind GR3027's effects are still unclear [106]. However, there are conflicting pro- and anti-inflammatory data on GABA's effect, so the area is not thoroughly investigated yet [128–131]. ALLO has also been reported to have anti-inflammatory effects, but ALLO's results do not appear to be via GABA-A receptors and can also be activated by pregnenolone, which does not bind to the GABA-A receptor [132].

Clinical trials with GR3027 in HE

In awake humans, the effect of steroid-PAM can be examined with measurements of SEV [133]. GABA-A receptors minutely control SEV, an objective measure that is not affected by volition. SEV has often been used to determine the effect of sedative PAM on the individual and has, among other things, been used as evidence of driving under the drug influence. SEV can also be used to check the efficacy and sensitivity of steroid-PAMs such as ALLO. Using SEV and sedation estimates, we have shown that ALLO has a dose-dependent CNS effect that also shows a strong relationship with plasma concentrations [133]. We have used SEV and sedation to investigate different GABAergic antagonists—including new drugs against the overproduction or effect of endogenous steroid-PAMs, that is, GAMSA. For this purpose, we have synthesized oral substances, and one—GR3027, also called golexanolone—has a GABA-A receptor subunit profile that shows selectivity for the α5 receptor subtype, and thereby the treatment of cognitive and alertness impairment. Studies we

have done in rodents have shown that GR3027 can inhibit ALLO's cognitive-disrupting effects very effectively. We have therefore tested GR3027 through a toxicological procedure, which has shown that GR3027 is safe and does not show any serious side effects, and can be used as a drug against excessive GABAergic tone in the CNS. To demonstrate a target engagement of GR3027, we have used ALLO-induced reduction in SEV and an increase in sedation. We gave men GR3027 orally but ALLO intravenously at a previously tested dose in a placebo-controlled randomized crossover study. GR3027 treatment significantly reduced the ALLO effect in both SEV and sedation [28]. As mentioned above, HE is a disease that has a high GABAergic tone and high ALLO levels in the blood. We have conducted a clinical trial with GR3027 in adult patients with HE and cognitive impairment [134]. We conducted a double-blind randomized placebo-controlled trial with GR3027 (golexanolone) for 21 days of treatment. Outcome parameters were an objective measure measured with an electroencephalogram (EEG, theta frequency; delta + theta/alpha + beta DT/AB ratio) that can be used for diagnosis and measuring treatment effect in HE subjects. In addition, patients were asked to complete various tests for encephalopathy score, animal naming, continuous reaction time, and Epworth Sleepiness Scale. The tests were administered at baseline, and after 10 and 21 days of treatment. Compared to baseline measurements, the group receiving golexanolone treatment showed a significant improvement, relative to placebo, in EEG performance (by normalizing the theta frequency and the ratio of DT/AB frequencies), Epworth Sleepiness Scale, continuous reaction time, psychometric liver encephalopathy score, and animal naming [134]. Further studies are ongoing in patients with primary cholestasis.

Author contributions

Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—original draft; writing—review and editing: Torbjörn Bäckström. *Conceptualization; data curation; investigation; methodology; project administration; validation; visualization; writing—review and editing*: Sahruh Turkmen. *Conceptualization; data curation; investigation; methodology; supervision; validation; writing—review and editing*: Roshni Das. *Conceptualization; data curation; funding acquisition; investigation; methodology; supervision; validation; writing—review and editing*: Magnus Doverskog. *Conceptualization; data curation; funding acquisition; investigation; methodology; supervision; validation; writing—review and editing*: Thomas P. Blackburn.

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Conflict of interest statement

TB has shares in Umecrine AB and Umecrine Cognition AB. RD has been employed by Umecrine AB. MD and TPB are employed, or board member of Umecrine Cognition AB. ST and RD has no conflict of interest.

References

1 Alzheimer's Association. Alzheimer's disease facts and Figs. *Alzheimer's Dement.* 2017;**13**:325–73.
2 Langer K, Jentsch VL, Wolf OT. Rapid effects of acute stress on cognitive emotion regulation *Psychoneuroendocrinology.* 2023;**151**:106054
3 Tiihonen-Möller A, Backstrom T, Sondergaard HP, Helstrom L. Identifying risk factors for PTSD in women seeking medical help after rape. *PLoS One.* 2014;**9**(10):e111136.
4 McEwen BS. Central effects of stress hormones in health and disease: understanding the protective and damaging effects of stress and stress mediators. *Eur J Pharmacol.* 2008;**583**(2–3):174–85.
5 Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc Natl Acad Sci USA.* 1991;**88**(10):4553–7.
6 Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, et al. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. *J Neurochem.* 2000;**75**(2):732–40.
7 Bengtsson S, Bäckström T, Brinton R, Irwin R, Johansson M, Sjöstedt J, Wang M. $GABA_A$ receptor modulating steroids in acute and chronic stress; relevance for cognition and dementia. *Neurobiol Stress.* 2020;**12**:100206.. https://doi.org/10.1016/j.ynstr.2019.100206
8 Droogleever Fortuyn HA, van Broekhoven F, Verkes RJ, Bäckström T, Zitman FG, Span PN. Effects of PhD examination stress on allopregnanolone and cortisol plasma levels and peripheral benzodiazepine receptors. *Psychoneuroendocrinology.* 2004;**29**:1341–4.
9 Ossewaarde L, Hermans EJ, van Wingen GA, Kooijman SC, Johansson IM, Backstrom T, et al. Neural mechanisms underlying changes in stress-sensitivity across the menstrual cycle. *Psychoneuroendocrinology.* 2010;**35**(1):47–55.

10 Ossewaarde L, van Wingen GA, Rijpkema M, Backstrom T, Hermans EJ, Fernandez G. Menstrual cycle-related changes in amygdala morphology are associated with changes in stress sensitivity. *Hum Brain Mapp.* 2013;**34**(5):1187–93.
11 Fava GA, McEwen BS, Guidi J, Gostoli S, Offidani E, Sonino N. Clinical characterization of allostatic overload. *Psychoneuroendocrinology.* 2019;**108**:94–101.
12 Bixo M, Andersson A, Winblad B, Purdy RH, Bäckström T. Progesterone, 5α-pregnane-3,20-dione and 3αhydroxy-5α-pregnane-20-one in specific regions of the human female brain at different endocrine states. *Brain Res.* 1997;**764**:173–8.
13 Strömberg J, Haage D, Taube M, Bäckström T, Lundgren P. Neurosteroid modulation of allopregnanolone and GABA effect on the $GABA_A$ receptor. *Neuroscience.* 2006;**143**(1):73–81.
14 Lupien SJ, Fiocco A, Wan N, Maheu F, Lord C, Schramek T, et al. Stress hormones and human memory function across the lifespan. *Psychoneuroendocrinology.* 2005;**30**(3):225–42
15 Marin MF, Lord C, Andrews J, Juster RP, Sindi S, Arsenault-Lapierre G, et al. Chronic stress, cognitive functioning and mental health. *Neurobiol Learn Mem.* 2011;**96**(4):583–95.
16 Yaffe K, Vittinghoff E, Lindquist K, Barnes D, Covinsky KE, Neylan T, et al. Posttraumatic stress disorder and risk of dementia among US veterans. *Arch Gen Psychiatry.* 2010;**67**(6):608–13.
17 Pisu MG, Concas L, Sidi C, Serra M, Porcu P. The allopregnanolone response to acute stress in females: preclinical and clinical studies. *Biomolecules.* 2022;**12**:1262. https://doi.org/10.3390/biom12091262
18 Johansson L, Guo X, Waern M, Östling S, Gustafson D, Bengtsson C, et al. Midlife psychological stress and risk of dementia: a 35-year longitudinal population study. *Brain.* 2010;**133**(8):2217–24.
19 Wang H-X, Wahlberg M, Karp A, Winblad B, Fratiglioni L. Psychosocial stress at work is associated with increased dementia risk in late life. *Alzheimer's Dement.* 2012;**8**(2):114–20.
20 Sindi S, Hagman G, Hakansson K, Kulmala J, Nilsen C, Kareholt I, et al. Midlife work-related stress increases dementia risk in later life: the CAIDE 30-year study. *J Gerontol B Psychol Sci Soc Sci.* 2017;**72**(6):1044–53.
21 Jeong YH, Park CH, Yoo J, Shin KY, Ahn S-M, Kim H-S, et al. Chronic stress accelerates learning and memory impairments and increases amyloid deposition in APPV717I-CT100 transgenic mice, an Alzheimer's disease model. *FASEB J.* 2006;**20**(6):729–51.
22 Tran TT, Srivareerat M, Alkadhi KA. Chronic psychosocial stress accelerates impairment of long-term memory and late-phase long-term potentiation in an at-risk model of Alzheimer's disease. *Hippocampus.* 2010;**21**(7):724–32.
23 Machado A, Herrera AJ, de Pablos RM, Espinosa-Oliva AM, Sarmiento M, Ayala A, et al. Chronic stress as a risk factor for Alzheimer's disease. *Rev Neurosci.* 2014;**25**(6):785–804.
24 Rasmusson AM, Novikov O, Brown KD, Pinna G, Pineles SL. Pleiotropic endophenotypic and phenotype effects of GABAergic neurosteroid synthesis deficiency in post-traumatic stress disorder. *Curr Opin Endocr Metab Res.* 2022;**25**:100359. https://doi.org/10.1016/j.coemr.2022.100359
25 Sandstrom A, Rhodin IN, Lundberg M, Olsson T, Nyberg L. Impaired cognitive performance in patients with chronic burnout syndrome. *Biol Psychol.* 2005;**69**(3):271–9.
26 Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA(A) receptors. *Neuropharmacology.* 2002;**43**(4):651–61.
27 Timby E, Bäckström T, Nyberg S, Stenlund H, Wihlbäck A-C, Bixo M. Women with premenstrual dysphoric disorder have altered sensitivity to allopregnanolone over the menstrual cycle compared to controls—a pilot study. *Psychopharmacology (Berl).* 2016;**233**(11):2109–17.
28 Johansson M, Månsson M, Lins LE, Scharschmidt B, Doverskog M, Bäckström T. GR3027 reversal of neurosteroid-induced, $GABA_A$ receptor-mediated inhibition of human brain function: an allopregnanolone challenge study. *Psychopharmacology (Berl).* 2018;**235**(5):1533–43
29 Möller AT, Backstrom T, Nyberg S, Sondergaard HP, Helstrom L. Women with PTSD have a changed sensitivity to $GABA_A$ receptor active substances. *Psychopharmacology (Berl).* 2016;**233**(11):2025–33.
30 Bäckström T, Bixo M, Nyberg S, Savic I. Increased neurosteroid sensitivity—an explanation to symptoms associated with chronic work-related stress in women? *Psychoneuroendocrinology.* 2013;**38**(7):1078–89
31 Birzniece V, Bäckström T, Johansson IM, Lindblad C, Lundgren P, Löfgren M, et al. Neuroactive steroid effects on cognitive functions with a focus on the serotonin and GABA systems. *Brain Res Rev.* 2006;**51**(2):212–39.
32 De Koninck Y. Altered chloride homeostasis in neurological disorders: a new target. *Curr Opin Pharmacol.* 2007;**7**:93–9.
33 Pozzi D, Rasile M, Corradini I, Matteoli M. Environmental regulation of the chloride transporter KCC2: switching inflammation off to switch the GABA on? *Transl Psychiatry.* 2020;**349**:1–11. https://doi.org/10.1038/s41398-020-01027-6
34 Bäckström T, Haage D, Löfgren M, Johansson IM, Strömberg J, Nyberg S. et al. Paradoxical effects of GABA-A modulators may explain sex steroid induced negative mood symptoms in some persons. *Neuroscience.* 2011;**191**:46–54
35 Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci.* 2005;**6**(7):565–75.
36 Barnard EA, Skolnick P, Olsen RW, Mohler H, Sieghart W, Biggio G, et al. International Union of Pharmacology. XV. Subtypes of γ-aminobutyric AcidA receptors: classification on the basis of subunit structure and receptor function. *Pharmacol Rev.* 1998;**50**(2):291–314.
37 Whiting PJ, Bonnert TP, McKernan RM, Farrar S, Bourdelles BL, Heavens RP, et al. Molecular and functional diversity of the expanding $GABA_A$ receptor gene family. *Ann NY Acad Sci.* 1999;**868**:645–53.
38 Olsen RW, Sieghart W. GABA A receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology.* 2009;**56**(1):141–8.
39 Korpi ER, Grunder G, Luddens H. Drug interactions at GABA(A) receptors. *Prog Neurobiol.* 2002;**67**(2):113–59.
40 Locci A, Pinna G. Neurosteroid biosynthesis down-regulation and changes in GABAA receptor subunit composition: a biomarker axis in stress-induced cognitive and emotional impairment. *Br J Pharmacol.* 2017;**174**(19):3226–41.

41 Macdonald RL, Olsen RW. GABAA receptor channels. *Annu Rev Neurosci.* 1994;**17**:569–602.
42 Sieghart W. GABAA receptors: ligand-gated Cl- ion channels modulated by multiple drug-binding sites. *Trends Pharmacol Sci.* 1992;**13**(12):446–50.
43 Smith MC, Riskin BJ. The clinical use of barbiturates in neurological disorders. *Drugs.* 1991;**42**(3):365–78.
44 Harris RA, Proctor WR, McQuilkin SJ, Klein RL, Mascia MP, Whatley V, et al. Ethanol increases GABAA responses in cells stably transfected with receptor subunits. *Alcohol Clin Exp Res.* 1995;**19**(1):226–32.
45 Smart TG. A novel modulatory binding site for zinc on the GABAA receptor complex in cultured rat neurones. *J Physiol.* 1992;**447**:587–625.
46 Hawkinson JE, Kimbrough CL, Belelli D, Lambert JJ, Purdy RH, Lan NC. Correlation of neuroactive steroid modulation of [35S]t-butylbicyclophosphorothionate and [3H]flunitrazepam binding and gamma-aminobutyric acidA receptor function. *Mol Pharmacol.* 1994;**46**(5):977–85.
47 Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, et al. Neurosteroids act on recombinant human GABAA receptors. *Neuron.* 1990;**4**(5):759–65.
48 Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science.* 1986;**232**(4753):1004–7.
49 Lambert JJ, Belelli D, Harney SC, Peters JA, Frenguelli BG. Modulation of native and recombinant GABA(A) receptors by endogenous and synthetic neuroactive steroids. *Brain Res Brain Res Rev.* 2001;**37**(1–3):68–80.
50 Frye CA, Van Keuren KR, Erskine MS. Behavioral effects of 3 alpha-androstanediol. I: Modulation of sexual receptivity and promotion of GABA-stimulated chloride flux. *Behav Brain Res.* 1996;**79**(1–2):109–18.
51 Norberg L, Wahlstrom G, Backstrom T. The anaesthetic potency of 3 alpha-hydroxy-5 alpha-pregnan-20-one and 3 alpha-hydroxy-5 beta-pregnan-20-one determined with an intravenous EEG-threshold method in male rats. *Pharmacol Toxicol.* 1987;**61**(1):42–7.
52 Reddy DS, Jian K. The testosterone-derived neurosteroid androstanediol is a positive allosteric modulator of GABAA receptors. *Pharmacol Exp Ther.* 2010;**334**(3):1031–41. https://doi.org/10.1124/jpet.110.169854
53 Landgren S, Aasly J, Bäckström T, Dubrowsky B, Danielsson E. The effect of progesterone and its metabolites on the interictal epileptiform discharge in the cat's cortex. *Acta Physiol Scand.* 1987;**131**:33–42.
54 Landgren S, Wang MD, Backstrom T, Johansson S. Interaction between 3 alpha-hydroxy-5 alpha-pregnan-20-one and carbachol in the control of neuronal excitability in hippocampal slices of female rats in defined phases of the oestrus. *Acta Physiol Scand.* 1998;**162**(1):77–88
55 Johansson IM, Birzniece V, Lindblad C, Olsson T, Backstrom T. Allopregnanolone inhibits learning in the Morris water maze. *Brain Res.* 2002;**934**(2):125–31.
56 Mayo W, Dellu F, Robel P, Cherkaoui J, Le Moal M, Baulieu EE, et al. Infusion of neurosteroids into the nucleus basalis magnocellularis affects cognitive processes in the rat. *Brain Res.* 1993;**607**(1–2):324–8.
57 Kask K, Backstrom T, Nilsson LG, Sundstrom-Poromaa I. Allopregnanolone impairs episodic memory in healthy women. *Psychopharmacology (Berl).* 2008;**199**(2):161–8.
58 Perry RJ, Hodges JR. Spectrum of memory dysfunction in degenerative disease. *Curr Opin Neurol.* 1996;**9**(4):281–5.
59 Dubrovsky B, Tatarinov-Levin A, Harris J. Effects of the active neurosteroid allotetrahydrodeoxycorticosterone on long-term potentiation in the rat hippocampus: implications for depression. *Prog Neuropsychopharmacol Biol Psychiatry.* 2004;**28**(6):1029–34.
60 George O, Vallée M, Le Moal M, Mayo W. Neurosteroids and cholinergic systems: implications for sleep and cognitive processes and potential role of age-related changes. *Psychopharmacology (Berl).* 2006;**186**(3):402.
61 van Wingen G, van Broekhoven F, Verkes RJ, Petersson KM, Backstrom T, Buitelaar J, et al. How progesterone impairs memory for biologically salient stimuli in healthy young women. *J Neurosci.* 2007;**27**(42):11416–23.
62 Barker MJ, Greenwood KM, Jackson M, Crowe SF. Persistence of cognitive effects after withdrawal from long-term benzodiazepine use: a meta-analysis. *Arch Clin Neuropsychol.* 2004;**19**(3):437–54.
63 Saunders PA, Copeland JR, Dewey ME, Davidson IA, McWilliam C, Sharma V, et al. Heavy drinking as a risk factor for depression and dementia in elderly men. Findings from the Liverpool longitudinal community study. *Br J Psychiatry.* 1991;**159**:213–6.
64 Penninkilampi R, Eslick GD. A systematic review and meta-analysis of the risk of dementia associated with benzodiazepine use, after controlling for protopathic bias. *CNS Drugs.* 2018;**32**(6):485–97.
65 Mohammed AK, Wahlstrom G, Tiger G, Bjorklund PE, Stenstrom A, Magnusson O, et al. Impaired performance of rats in the Morris swim-maize test late in abstinence following long-term sodium barbital treatment. *Drug Alcohol Depend.* 1987;**20**(3):203–12.
66 Taipale H, Gomm W, Broich K, Maier W, Tolppanen AM, Tanskanen A, et al. Use of antiepileptic drugs and dementia risk—an analysis of Finnish health register and German health insurance data. *J Am Geriatr Soc.* 2018;**66**(6):1123–9.
67 Park SP, Kwon SH. Cognitive effects of antiepileptic drugs. *J Clin Neurol.* 2008;**4**(3):99–106.
68 Yaffe K, Sawaya G, Lieberburg I, Grady D. Estrogen therapy in postmenopausal women: effects on cognitive function and dementia. *JAMA.* 1998;**279**:688–95.
69 LeBlanc ES, Janowsky J, Chan BK, Nelson HD. Hormone replacement therapy and cognition: systematic review and meta-analysis. *JAMA.* 2001;**285**:1489–99.
70 Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, et al. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA.* 2003;**289**(20):2651–62.
71 Shumaker SA, Legault C, Kuller L, Rapp SR, Thal L, Lane DS, et al. Conjugated equine estrogens and incidence of probable dementia and mild cognitive impairment in postmenopausal women. *JAMA.* 2004;**291**(24):2947–58.
72 Coker LH, Hogan PE, Bryan NR, Kuller LH, Margolis KL, Bettermann K, et al. Postmenopausal hormone therapy and subclinical cerebrovascular disease. *Neurology.* 2009;**72**(2):125–34.
73 Coker LH, Espeland MA, Rapp SR, Legault C, Resnick SM, Hogan P, et al. Postmenopausal hormone therapy and

cognitive outcomes: the Women's Health Initiative Memory Study (WHIMS). *J Steroid Biochem Mol Biol.* 2010;**118**(4–5):304–10.
74 Honjo H, Iwasa K, Kawata M, Fushiki S, Hosoda T, Tatsumi H, et al. Progestins and estrogens and Alzheimer's disease. *J Steroid Biochem Mol Biol.* 2005;**93**(2–5):305–8.
75 Braden B, Talboom JS, Crain ID, Simard AR, Lukas RJ, Prokai L, et al. Medroxyprogesterone acetate impairs memory and alters the GABAergic system in aged surgically menopausal rats. *Neurobiol Learn Mem.* 2010;**93**(3):444–53.
76 Braden B, Garcia A, Mennenga S, Prokai L, Villa S, Acosta J, et al. Cognitive-impairing effects of medroxyprogesterone acetate in the rat: independent and interactive effects across time. *Psychopharmacology (Berl).* 2011;**218**(2):405–18.
77 Lowry NC, Pardon LP, Yates MA, Juraska JA. Effects of long-term treatment with 17 β-estradiol and medroxyprogesterone acetate on water maze performance in middle-aged female rats. *Horm Behav.* 2010;**58**:200–7.
78 Meyerson B. Relationship between the anesthetic and gestagenic action and estrous behavior-inducing activity of different progestins. *Endocrinology.* 1967;**81**(2):369–74.
79 Bixo M, Bäckström T. Regional distribution of progesterone and 5a-pregnane-3,20-dione in rat brain during progesterone induced anesthesia. *Psychoneuroendocrinology.* 1990;**15**:159–62
80 Das R, Ragagnin G, Sjöstedt J, Johansson M, Haage D, Druzin M, et al. Medroxyprogesterone acetate positively modulates specific $GABA_A$-receptor subtypes—affecting memory and cognition. *Psychoneuroendocrinology.* 2022;**141**:105754.
81 Olsen RW, Sieghart W. International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric acidA receptors: classification on the basis of subunit composition, pharmacology, and function. *Pharmacol Rev.* 2008;**60**:243–60. https://doi.org/10.1124/pr.108.00505
82 Ghit A, Assal D, Al-Shami AS, Hussein DEE. GABAA receptors: structure, function, pharmacology, and related disorders. *J Genet Eng Biotechnol.* 2021;**19**:123. https://doi.org/10.1186/s43141-021-00224-0
83 Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, et al. Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the $GABA_A$ receptor. *J Neurosci.* 2002;**22**(13):5572–80.
84 Maubach K. GABA(A) receptor subtype selective cognition enhancers. *Curr Drug Targets CNS Neurol Disord.* 2003;**2**(4):233–9.
85 Löw K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, et al. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science.* 2000;**290**:131–4.
86 Bäckström T, Zetterlund B, Blom S, Romano M. Effects of continuous progesterone infusion on the epileptic discharge frequency in women with partial epilepsy. *Acta Neurol Scand.* 1984;**69**:240–8
87 Kokate TG, Banks MK, Magee T, Yamaguchi S, Rogawski MA. Finasteride, a 5alpha-reductase inhibitor, blocks the anticonvulsant activity of progesterone in mice. *J Pharmacol Exp Ther.* 1999;**288**(2):679–84.
88 Löfgren M, Holmberg E, Bäckström T, Egecioglu E, Dickson SL. The additive effect of allopregnanolone on ghrelin's orexigenic effect in rats. *Neuropeptides.* 2019;**76**:e101937. https://doi.org/10.1016/j.npep.2019.101937
89 Astur RS, Taylor LB, Mamelak AN, Philpott L, Sutherland RJ. Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. *Behav Brain Res.* 2002;**132**(1):77–84.
90 Farr SA, Flood JF, Morley JE. The effect of cholinergic, GABAergic, serotonergic, and glutamatergic receptor modulation on posttrial memory processing in the hippocampus. *Neurobiol Learn Mem.* 2000;**73**(2):150–67.
91 Morris RGM, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature.* 1986;**319**:774–6.
92 Bengtsson SK, Johansson M, Bäckström T, Wang M. Chronic allopregnanolone treatment accelerates Alzheimer's disease development in AβPPSwePSEN1ΔE9 mice. *J Alzheimers Dis.* 2012;**31**(1):71–84.
93 Bengtsson SK, Johansson M, Bäckström T, Nitsch RM, Wang M. Brief but chronic increase in allopregnanolone causes accelerated AD pathology differently in two mouse models. *Curr Alzheimer Res.* 2013;**10**(1):38–47.
94 Chen S, Wang JM, Irwin RW, Yao J, Liu L, Brinton RD. Allopregnanolone promotes regeneration and reduces β-amyloid burden in a preclinical model of Alzheimer's disease. *PLoS One.* 2011;**6**(8):e24293.
95 Irwin RW, Brinton RD. Allopregnanolone as regenerative therapeutic for Alzheimer's disease: translational development and clinical promise. *Prog Neurobiol.* 2014;**113**:40–55.
96 Bengtsson SK, Johansson M, Bäckström T. Long-term continuous allopregnanolone elevation causes memory decline and hippocampus shrinkage, in female wildtype B6 mice. *Horm Behav.* 2016;**78**:160–7.
97 Turkmen S, Bäckström T, Wahlstrom G, Andreen L Johansson IM. Tolerance to allopregnanolone with focus on the GABA-A receptor. *Br J Pharmacol.* 2011;**162**:311–27
98 Ahboucha S, Gamrani H, Baker G. GABAergic neurosteroids: the "endogenous benzodiazepines" of acute liver failure. *Neurochem Int.* 2012;**60**(7):707–14.
99 Felipo V. Hepatic encephalopathy: effects of liver failure on brain function. *Nat Rev Neurosci.* 2013;**14**(12):851–8.
100 Sergeeva OA. GABAergic transmission in hepatic encephalopathy. *Arch Biochem Biophys.* 2013;**536**(2):122–30.
101 Jones EA. Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. *Metab Brain Dis.* 2002;**17**(4):275–81.
102 Ahboucha S, Pomier-Layrargues G, Mamer O, Butterworth RF. Increased levels of pregnenolone and its neuroactive metabolite allopregnanolone in autopsied brain tissue from cirrhotic patients who died in hepatic coma. *Neurochem Int.* 2006;**49**(4):372–8.
103 Norenberg MD, Itzhak Y, Bender AS. The peripheral benzodiazepine receptor and neurosteroids in hepatic encephalopathy. *Adv Exp Med Biol.* 1997;**420**:95–111.
104 Lavoie J, Layrargues GP, Butterworth RF. Increased densities of peripheral-type benzodiazepine receptors in brain autopsy samples from cirrhotic patients with hepatic encephalopathy. *Hepatology.* 1990;**11**(5):874–8.
105 Liu GJ, Middleton RJ, Hatty CR, Kam WW, Chan R, Pham T, et al. The 18 kDa translocator protein, microglia and neuroinflammation. *Brain Pathol.* 2014;**24**(6):631–53. https://doi.org/10.1111/bpa.12196

106 Mincheva G, Gimenez-Garzo C, Izquierdo-Altarejos P, Martinez-Garcia M, Doverskog M, Blackburn TP, et al. Golexanolone, a $GABA_A$ receptor modulating steroid antagonist, restores motor coordination and cognitive function in hyperammonemic rats by dual effects on peripheral inflammation and neuroinflammation. *CNS Neurosci Ther.* 2022;**28**(11):1861–74. https://doi.org/10.1111/cns.13926

107 Guilarte TR, Rodichkin AN, McGlothan JL, Acanda De La Rocha AM, Azzam DJ. Imaging neuroinflammation with TSPO: a new perspective on cellular sources and subcellular localization. *Pharmacol Ther.* 2022;**234**:108048. https://doi.org/10.1016/j.pharmthera.2021.108048

108 Itzhak Y, Roig-Cantisano A, Dombro RS, Norenberg MD. Acute liver failure and hyperammonemia increase peripheral-type benzodiazepine receptor binding and pregnenolone synthesis in mouse brain. *Brain Res.* 1995;**705**(1–2):345–8.

109 Butterworth RF. Altered glial-neuronal crosstalk: cornerstone in the pathogenesis of hepatic encephalopathy. *Neurochem Int.* 2010;**57**(4):383–8. https://doi.org/10.1016/j.neuint.2010.03.012

110 Wetten A, Ogle L, Mells G, Hegade VS, Jopson L, Corrigan M, et al. Neurosteroid activation of $GABA_A$ receptors: a potential treatment target for symptoms in primary biliary cholangitis? *Can J Gastroenterol Hepatol.* 2022;**2022**:1–13.[eCollection 2022.PMID: 36523650]. https://doi.org/10.1155/2022/3618090

111 Holmberg E, Sjöstedt J, Malinina E, Johansson M, Turkmen S, Ragagnin G, et al. Allopregnanolone involvement in feeding regulation, overeating and obesity. *Front Neuroendocrinol.* 2018;**48**:70–7. https://doi.org/10.1016/j.yfrne.2017.07.002

112 Bäckström T, Bixo M, Johansson M, Nyberg S, Ossewaarde L, Ragagnin G, et al. Allopregnanolone and mood disorders. *Prog Neurobiol.* 2014;**113**:88–94.

113 Hosie AM, Wilkins ME, da Silva HMA, Smart TG. Endogenous neurosteroids regulate GABAA receptors through two discrete transmembrane sites. *Nature.* 2006;**444**(7118):486–9.

114 Gyermek L, Iriarte J, CCCX CP. Structure-activity relationship of some steroidal hypnotic agents. *J Med Chem.* 1968;**11**:117–25

115 Bäckström T, Wahlström G, Wahlström K, Zhu D, Wang MD. Isoallopregnanolone; an antagonist to the anaesthetic effect of allopregnanolone in male rats. *Eur J Pharmacol.* 2005;**512**:15–21.

116 Wang MD, Bäckström T, Landgren S. The inhibitory effects of allopregnanolone and pregnanolone on the population spike, evoked in the rat hippocampal CA1 stratum pyramidale in vitro, can be blocked selectively by epiallopregnanolone. *Acta Physiol Scand.* 2000;**169**(4):333–41

117 Lundgren P, Strömberg J, Bäckström T, Wang MD. Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3β-hydroxy-5α-pregnan-20-one (isoallopregnanolone). *Brain Res.* 2003;**982**:45–53.

118 Turkmen S, Lundgren P, Birzniece V, Zingmark E, Backstrom T, Johansson IM. 3beta-20beta-dihydroxy-5alpha-pregnane (UC1011) antagonism of the GABA potentiation and the learning impairment induced in rats by allopregnanolone. *Eur J Neurosci.* 2004;**20**:1604–12.

119 Vallee M, Mayo W, Koob GF, Le Moal M. Neurosteroids in learning and memory processes. *Int Rev Neurobiol.* 2001;**46**:273–320.

120 Seljeset S, Liebowitz S, Bright DP, Smart TG. Pre- and postsynaptic modulation of hippocampal inhibitory synaptic transmission by pregnenolone sulphate. *Neuropharmacology.* 2023;**233**:109530. https://doi.org/10.1016/j.neuropharm.2023.109530

121 Williamson J, Mtchedlishvili Z, Kapur J. Characterization of the convulsant action of pregnenolone sulfate. *Neuropharmacology.* 2004;**46**(6):856–64.

122 Johansson M, Agusti A, Llansola M, Montoliu C, Strömberg J, Malinina E, et al. GR3027 antagonizes GABAA receptor-potentiating neurosteroids and restores spatial learning and motor coordination in rats with chronic hyperammonemia and hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol.* 2015;**309**:G400–9. https://doi.org/10.1152/ajpgi.00073

123 Cabrera-Pastor A, Balzano T, Hernández-Rabaza V, Malaguarnera M, Llansola M, Felipo V. Increasing extracellular cGMP in cerebellum in vivo reduces neuroinflammation, GABAergic tone and motor in-coordination in hyperammonemic rats. *Brain Behav Immun.* 2017;**69**:386–98.

124 Cabrera-Pastor A, Llansola M, Montoliu C, Malaguarnera M, Balzano T, Taoro-Gonzalez L, et al. Peripheral inflammation induces neuroinflammation that alters neurotransmission and cognitive and motor function in hepatic encephalopathy: underlying mechanisms and therapeutic implications. *Acta Physiol.* 2019;**226**(2):e13270.

125 Arenas YM, Balzano T, Ivaylova G, Llansola M, Felipo V. The S1PR2-CCL2-BDNF-TrkB pathway mediates neuroinflammation and motor incoordination in hyperammonaemia. *Neuropathol Appl Neurobiol.* 2022;**48**:e12799.

126 Cauli O, Mansouri MT, Agusti A, Felipo V. Hyperammonemia increases GABAergic tone in the cerebellum but decreases it in the rat cortex. *Gastroenterology.* 2009;**136**(4):1359–67

127 Ahboucha S, Jiang W, Chatauret N, Mamer O, Baker GB, Butterworth RF. Indomethacin improves locomotor deficit and reduces brain concentrations of neuroinhibitory steroids in rats following portacaval anastomosis. *Neurogastroenterol Motil.* 2008;**20**(8):949–57.

128 Crowley T, Cryan JF, Downer EJ, O'Leary OF. Inhibiting neuroinflammation: the role and therapeutic potential of GABA in neuro-immune interactions. *Brain Behav Immun.* 2016;**54**:260–77.

129 Tian J, Chau C, Hales TG, Kaufman DL. GABA(A) receptors mediate inhibition of T cell responses. *J Neuroimmunol.* 1999;**96**(1):21–8.

130 Zhang L, Tan J, Jiang X, Qian W, Yang T, Sun X, et al. Neuron-derived CCL2 contributes to microglia activation and neurological decline in hepatic encephalopathy. *Biol Res.* 2017;**50**(1):1–11.

131 Guyon A. CXCL12 chemokine and GABA neurotransmitter systems crosstalk and their putative roles. *Front Cell Neurosci.* 2014;**5**:1–7.

132 Balan I, Beattie MC, O'Buckley TK, Aurelian L, Morrow AL. Endogenous neurosteroid 3α,5α-hydroxypregnan-20-one inhibits toll-like-4 receptor activation and pro-inflammatory signaling in macrophages and brain. *Sci Rep.* 2019;**9**(1):1–14.

133 Timby E, Balgård M, Nyberg S, Spigset O, Andersson A, Porankiewicz-Asplund J, et al. Pharmacodynamic effects of allopregnanolone in healthy women. *Psychopharmacology (Berl).* 2006;**186**(3):414–24.
134 Montagnese S, Lauridsen M, Vilstrup H, Zarantonello L, Lakner G, Fitilev S, et al. A pilot study of golexanolone, a new $GABA_A$ receptor modulating steroid antagonist, in patients with covert hepatic encephalopathy. *J Hepatol.* 2021;**75**(1):98–107. https://doi.org/10.1016/j.jhep.2021.03.012
135 Carl P, Högskilde S, Nielsen JW, Sörensen MB, Lindholm M, Karlen B, et al. Pregnanolone emulsion: A preliminary pharmacokinetic and pharmacodynamic study of a new intravenous anaesthetic agent. *Anaesthesia.* 1990;**45**:189–97.
136 Meltzer-Brody S, Colquhoun H, Riesenberg R, Epperson CN, Deligiannidis KM, Rubinow DR. et al. Brexanolone injection in post-partum depression: two multicentre, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet.* 2018;**392**:1058–70.
137 Brinton RD. Neurosteroids as regenerative agents in the brain: therapeutic implications. *Nat Rev Endocrinol.* 2013;**9**(4):241–50.
138 Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure-activity relationships for steroid interaction with the GABA-A receptor complex. *J Pharmacol Exp Ther.* 1987;**241**(1):346–53.

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