Published in final edited form as:
J Neuroendocrinol. 2012 January ; 24(1): 102–116. doi:10.1111/j.1365-2826.2011.02234.x.

Upregulation of neurosteroid biosynthesis as a pharmacological strategy to improve behavioral deficits in a putative mouse model of PTSD

Graziano Pinna[1] and **Ann M Rasmusson**[2]

[1]Psychiatric Institute, Department of Psychiatry, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612

[2]VA Boston Healthcare System; National Center for Posttraumatic Stress Disorder, Women's Health Science Division; Boston University School of Medicine, Boston, MA 02130

Abstract

Benzodiazepines remain the most frequently used psychotropic drugs for the treatment of anxiety spectrum disorders; however their use is associated with development of tolerance and dependence. Another major hindrance is represented by their lack of efficacy in many patients, including patients with posttraumatic stress disorder (PTSD). For these non-responders, the use of selective serotonin reuptake inhibitors (SSRIs) has been the therapy of choice.

In the past decade, clinical studies have suggested that the pharmacological action of SSRIs may include the ability of these drugs to normalize decreased brain levels of neurosteroids in patients with depression and PTSD, in particular the progesterone derivative allopregnanolone, which potently and allosterically modulates the action of GABA at $GABA_A$ receptors.

Preclinical studies using the socially isolated mouse as an animal model of PTSD have demonstrated that fluoxetine and congeners ameliorate anxiety-like behavior, fear responses, and aggressive behavior expressed by such mice by increasing corticolimbic levels of allopregnanolone. This is a novel and more selective mechanism than 5-HT reuptake inhibition, which for half a century has been thought to be the main molecular mechanism for the therapeutic action of SSRIs. Importantly, this finding may shed light on the high rates of SSRI resistance among patients with PTSD and depression, disorders in which there appears to be a block in allopregnanolone synthesis. There are several different mechanisms by which such a block may occur, and SSRIs may only be corrective under some conditions. Thus, upregulation of allopregnanolone biosynthesis in corticolimbic neurons may offer a novel non-traditional pharmacological target for a new generation of potent non-sedating, anxiolytic medications for the treatment of anxiety, depression, and PTSD: selective brain steroidogenic stimulants (SBSSs).

Keywords

Allopregnanolone; 5α-reductase type I; selective brain steroidogenic stimulants (SBSSs); aggressive behavior; $GABA_A$ receptors; social isolation; anxiety; PTSD

Address for correspondence: Graziano Pinna, PhD, The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, 1601 W. Taylor Street, Chicago IL 60612, Tel.: 312-355-1464, Fax: 312-413-4569, gpinna@psych.uic.edu.

Introduction

Anxiety spectrum disorders, including generalized anxiety, panic, and posttraumatic stress disorder (PTSD) are debilitating conditions that affect 8–13% of the world's population and they are part or often associated with other psychiatric conditions such as depression (1–3). The best and most used treatments for these conditions are the anxiolytic benzodiazepines, including diazepam and alprazolam (4–7). Therapeutic use of these drugs is however associated with unwanted side effects such as sedation and rapid development of tolerance and physical dependence, which results in severe withdrawal symptoms following their discontinuation and may eventually lead to drug abuse (4–6; 8; 9).

Notwithstanding the widespread use of these drugs, another major drawback is their lack of efficacy in many patients, including patients with PTSD (10–12). Hence, for many decades drug design for anxiety disorders has focused on the development and testing of rapidly acting molecules with strong anxiolytic effects but possibly devoid of sedative effects, as well as tolerance and withdrawal liabilities (13–15). This has led investigators to search novel neuronal biomarkers as the pharmacological target for the next generation of anxiolytic drugs.

During the last decade, the downregulation of neurosteroid biosynthesis has been implicated as a possible contributor to the development of anxiety and depressive disorders (reviewed in 16). Clinical studies demonstrated that decreases in the serum/plasma and CSF content of neuroactive steroids, particularly the $GABA_A$ receptor-active progesterone derivative, allopregnanolone, are associated with emotional disorders, including depression, anxiety spectrum disorders, PTSD, premenstrual dysphoric disorder, schizophrenia, and impulsive aggression (17–27). These observations led to the hypothesis that downregulation of brain allopregnanolone content could be a cause or risk factor for these psychiatric disorders, and stimulated several research groups worldwide to develop new molecules to stimulate neurosteroidogenesis and allopregnanolone biosynthesis, in particular, in order to treat anxiety disorder and depression (28–32).

In our laboratory, we measured allopregnanolone levels in the cerebrospinal fluid (CSF) of patients with psychiatric disorders assuming that allopregnanolone levels in the CSF reflect the levels of this neurohormone in the brain (17). In depressed patients, the concentration of allopregnanolone in the CSF was approximately half of that measured in the CSF of nonpsychiatric patients (26). To test the hypothesis that this decrease in the CSF allopregnanolone levels of depressed patients reflects a decrease of brain allopregnanolone content, we compared the expression of 5α-reductase type I mRNA in the prefrontal-cortex (area BA9) between depressed patients and age- and sex-matched non-psychiatric subjects (33). In depressed patients, the cortical level of 5α-reductase mRNA was dramatically decreased to about 50% of the levels measured in non-psychiatric comparison subjects (33).

Interestingly, treatment with fluoxetine and fluvoxamine normalized the CSF allopregnanolone content of the depressed patients studied (26). Moreover, there was a statistically significant correlation between the improvement in depressive symptoms and the increase of CSF allopregnanolone elicited by fluoxetine or fluvoxamine. Similar results were reported when allopregnanolone or levels of 5α-tetrahydrodeoxycorticosterone, another positive modulator of $GABA_A$ receptor function, were measured in the plasma of depressed patients treated with SSRIs (34). In a more recent human study, CSF allopregnanolone levels in premenopausal women with PTSD were 40% of levels seen in healthy comparison subjects and were inversely correlated with PTSD re-experiencing and comorbid depressive symptoms (17). In fact, CSF allopregnanolone levels were lowest in those patients with PTSD and comorbid depression. In addition, the *ratio* of

allopregnanolone to its steroid precursor, 5α-dihydroprogesterone, was decreased among the PTSD patients, suggesting the presence of a block in allopregnanolone synthesis (17). Taken together, these data suggest that a deficit of GABAergic neurotransmission, likely caused by a downregulation of brain allopregnanolone biosynthesis, must be among the molecular mechanisms considered in the etiology of PTSD.

Therefore, given the seminal discovery that fluoxetine and its congeners increase the content of allopregnanolone in the CSF of depressed patients (26) and in neurons of various rodent brain areas (35), we hypothesized that normalization of brain allopregnanolone levels may underlie the therapeutic effects of selective serotonin reuptake inhibitors (SSRIs) in depression and PTSD. This hypothesis has since been supported by preclinical research using the socially isolated mouse as an animal model of behavioral deficits that resemble symptoms of human anxiety disorders and PTSD (36; 37; reviewed in 16; 38 and in 39). Corticolimbic allopregnanolone levels become markedly decreased in association with the development of anxiety-like behaviors, resistance to sedation, and extreme aggression in the socially isolated mouse (16; 36; 40). Administration of fluoxetine and its congeners prevents both the downregulation of allopregnanolone levels and the emergence of such behaviors. Further, the behavioral effects of fluoxetine and its congeners were found to be unrelated to the serotonin reuptake blocking activity for which this class of psychotropic drugs was developed (36; 37).

These data are thus in support of a novel mechanism whereby SSRIs acting as selective brain *steroidogenic* stimulants (SBSSs) increase brain corticolimbic allopregnanolone levels and improve behavioral deficits characteristic of PTSD, anxiety, and depression.

Neurosteroid physiology at $GABA_A$ receptors

Neurosteroids may be synthesized in the brain independently from peripheral sources (41–45) and reach physiologically relevant levels that modulate gene expression and/or neurotransmission (46–53). When administered systemically or intraventricularly, allopregnanolone exerts important pharmacological actions, including anticonvulsant, anxiolytic, antidepressant, and at the highest dosage, sedative-hypnotic actions (54–61). All of these pharmacological actions are also elicited by other positive allosteric modulators of GABA action at $GABA_A$ receptors such as barbiturates and benzodiazepines (53; 62; 63). This posed the question as to whether pharmacological actions of allopregnanolone were exerted by the binding of allopregnanolone to $GABA_A$ receptors and allosteric modulation of the action of GABA at $GABA_A$ receptors. Indeed, application of allopregnanolone resulted in a potent (nM affinity) positive allosteric modulation of the action of GABA at $GABA_A$ receptors (46–47; 50; 51). The finding that allopregnanolone permits, facilitates, and fine-tunes the efficacy of direct $GABA_A$ receptor activators and positive allosteric modulators of GABA effects at $GABA_A$ receptors substantiates its endogenous physiological relevance (44; 48; 49; 64).

More recently, Hosie and collaborators demonstrated that allopregnanolone potentiates GABA responses via two binding sites in the $GABA_A$ receptor that, respectively, mediate the potentiation and direct activation effects of allopregnanolone. Direct $GABA_A$ receptor activation is initiated by binding of allopregnanolone at a site formed by interfacial residues between the α and β subunits (65). Binding of allopregnanolone at the potentiation site located in a cavity within the α-subunit results in marked enhancement of $GABA_A$ receptor activation (65). Other studies in the field have reported that $GABA_A$ receptors incorporating α4, α6, and δ subunits in combination with γ and β subunits show higher affinity for allopregnanolone (66; 67). The affinity of allopregnanolone for these $GABA_A$ receptor subtypes is in the low nM range (46; 47). Altogether, allopregnanolone allosteric positive

modulation of the action of GABA at $GABA_A$ receptors shows a broad pharmacological profile that lacks subunit selectivity and is therefore much less selective than that of benzodiazepines, which fail to activate $GABA_A$ receptors containing α4 or α6 subunits (4; 46, 47; 68). These observations are of strong relevance for designing therapeutic strategies to overcome behavioral deficits resulting from $GABA_A$ receptor signal transduction deficits.

Neurosteroid biosynthesis

The localization of enzymes involved in allopregnanolone biosynthesis in the brain remained unclear until recent investigations in the mouse (69; 70; reviewed in 71). By combining *in situ* hybridization with immunohistochemistry, we not only clarified the neuronal lo calization of the neurosteroidogenic enzymes, 5α-reductase and 3α-HSD, but also the manner in which allopregnanolone acts within the neurons in which it is produced, and acts at local post- and extra-synaptic $GABA_A$ receptors after secretion (69; 71). Interestingly, our investigations failed to find any relevant expression of these two enzymes in GABAergic cortical interneurons or glial cells (69). Rather, 5α-reductase and 3α-HSD were shown to be highly expressed and colocalized in a region-specific way in primary GABAergic and glutamatergic neurons, including pyramidal neurons, granular cells, reticulo-thalamic neurons, medium spiny neurons of the striatum and nucleus accumbens, and Purkinje cells (69; 70). Thus it appears that allopregnanolone synthesized in glutamatergic cortical or hippocampal pyramidal neurons or in granular cells of the dentate gyrus may be secreted in: 1) a paracrine manner which would allow allopregnanolone to reach $GABA_A$ receptors located in the synaptic membranes of other cortical or hippocampal pyramidal neurons, or 2) an autocrine fashion which would allow allopregnanolone to act locally by binding post-synaptic or extra-synaptic $GABA_A$ receptors located on the same dendrites or cell bodies of the cortical or hippocampal pyramidal neuron in which it was produced (69). Alternatively, allopregnanolone might not be released, but may instead diffuse laterally into synaptosome membranes of the cell bodies or dendritic arborization of glutamatergic neurons in which it is produced to attain intracellular access to specific neurosteroid binding sites of $GABA_A$ receptors (69; 72). In the amygdala, for example, this would functionally baffle the effects of concomitant excitatory inputs to glutamatergic projection neurons during exposure to unconditioned stress during fear conditioning or to conditioned stressors during extinction.

Interestingly, allopregnanolone produced in primary output GABAergic neurons from the reticular thalamic nucleus may secrete allopregnanolone simultaneously with GABA, to concomitantly act at post-synaptic $GABA_A$ receptors inserted in glutamatergic thalamocortical neurons (73). Very similarly, allopregnanolone synthesized by striatal medium spiny GABAergic neurons and cerebellar Purkinje cells may activate post-synaptic $GABA_A$ receptors located on cell bodies or dendrites of neurons in the deep cerebellar nuclei (69; 71).

Clarifying where and how allopregnanolone is synthesized across several brain regions has been pivotal to our understanding the possible mechanisms by which allopregnanolone is secreted and acts at $GABA_A$ receptors. These studies underscore the functional role of allopregnanolone in maintaining the strength of GABAergic neurotransmission under physiological conditions and how deficits in allopregnanolone biosynthesis may result in dysfunctional behavior. These experiments have also helped to clarify the circuitry involved in regulating emotional behaviors associated with GABAergic neurotransmission dysfunction.

The social isolation mouse models aspects of PTSD

a. Downregulation of allopregnanolone biosynthesis

Impaired neurosteroidogenesis in socially isolated rodents has been associated with several behavioral deficits that resemble behavioral abnormalities observed in patients with anxiety/depressive disorders (31, 39, 74). Thus, we have used socially isolated mice as a model to study the behavioral responses elicited by treatment with SBSSs, including the SSRI antidepressants that possess a potent neurosteroidogenic activity selectively at low doses as a principal action. Also, the socially isolated mouse model provides an opportunity to study the molecular mechanisms that induce GABAergic neurotransmission dysfunction that results from downregulation of allopregnanolone (39). Our laboratory and others have indeed determined that exposure of mice or rats to protracted social isolation stress for 4–8 weeks induces a decrease in allopregnanolone levels in several corticolimbic structures as a result of a downregulation of the mRNA and protein expression of 5α-reductase type I (36; 74–76; reviewed in 39; 77). Indeed, socially isolated mice show a 70% reduction in the rate of allopregnanolone and 5α-DHP biosynthesis compared to group-housed mice (78; 36). It remains to be established whether the reduction of brain neurosteroids caused by social isolation is permanent once instated or whether group-housed conditions or neurosteroidogenic drugs may permanently reverse the effects of social isolation on neurosteroidogenesis and behavior.

The distribution and the content of 5α-DHP and allopregnanolone in various brain regions is not uniform (49; 79). In rodents, the olfactory bulb shows the highest concentrations of 5α-DHP and allopregnanolone followed by the frontal cortex, hippocampus, amygdala, striatum, and cerebellum (79). By combining data gathered using three distinct methods, we have quantified, and localized the brain region-specific downregulation of 5α-reductase in socially isolated mice (70; 79). The largest decrease of 5α-reductase was found in the amygdala and hippocampus, followed by the olfactory bulb and the frontal cortex (79). The expression of 5α-reductase failed to change in the cerebellum and striatum (79). *In situ* immunohistochemical studies have demonstrated that 5α-reductase is specifically decreased in cortical pyramidal neurons of layers V-VI, in hippocampal CA3 pyramidal neurons and glutamatergic granular cells of the dentate gyrus, and in the pyramidal-like neurons of the basolateral amygdala (70). However, 5α-reductase fails to change in GABAergic neurons of the reticular thalamic nucleus, central amygdala, cerebellum, and in the medium spiny neurons of the caudatus and putamen (70).

Similarly, using gas chromatography mass fragmentography to detect picomolar amount of allopregnanolone in discrete brain structure, we confirmed that the decrease of 5α-reductase in the above-mentioned brain areas resulted in a reduction of allopregnanolone levels (79). The reduction of 5α-reductase type I mRNA and protein in corticolimbic structures appeared to be a quite selective change in neurosteroidogenesis. In fact, social isolation failed to change the expression of 3α-HSD, the mRNA expression of diazepam binding inhibitor, and the expression of the 18 kDa translocase protein (TSPO), which is involved in the transport of cholesterol across the inner mitochondrial membrane and activation of neurosteroidogenesis (80). Thus, the downregulation of 5α-reductase appears to be the main factor responsible for the reduction of corticolimbic allopregnanolone levels and associated $GABA_A$ receptor signal transduction deficits of socially isolated mice.

b. Decreased pharmacological action of $GABA_A$ receptor ligands

Decreasing allopregnanolone biosynthesis as a result of social isolation stress or by inhibiting 5α-reductase with the potent competitive 5α-reductase inhibitor SKF 105,111 has proven to reduce the loss of righting reflexes induced by $GABA_A$ receptor active agents.

The effects of SKF on the muscimol-, pentobarbital-, benzodiazepine-, or alcohol-induced loss of righting reflex can be reversed by the systemic or intracerebroventricular administration of allopregnanolone (44; 49). Similarly, social isolation or SKF-induced decrease of allopregnanolone results in facilitation of the seizure activity induced by several drugs that decrease $GABA_A$ receptor function, such as picrotoxin (64). The increased susceptibility to picrotoxin-induced seizures can be reversed in SKF-treated or socially isolated mice by administration of allopregnanolone at doses that have virtually no effects on group-housed control mice (64). Given that seizure activity induced by kainic acid or strychnine in socially isolated mice is similar to that induced by these agents in group housed mice, we concluded that protracted social isolation or SKF treatment-induced allopregnanolone biosynthesis downregulation is the primary reason for the $GABA_A$ receptor signal transduction deficits observed in these mice.

c. Behavioral deficits resulting from allopregnanolone biosynthesis downregulation

Allopregnanolone has also emerged as an important biomarker of behavioral deficits induced in rodent models of depression and anxiety spectrum disorders (16; 36; 38; 39; 78). By using socially isolated mice or pharmacological manipulation to induce a downregulation of allopregnanolone biosynthesis, we have established a fundamental role for allopregnanolone in the regulation of anxiety-like and aggressive behavior, as well as contextual fear conditioning, (16; 38; 64; 79; 81). In mice socially isolated for a period of up to eight weeks, we have demonstrated a time-dependent increase in aggressive behavior over the first four weeks of isolation, accompanied by a time-dependent decrease of corticolimbic allopregnanolone levels (35). Similarly, socially isolated mice exposed a classical fear conditioning paradigm showed enhanced conditioned contextual, but not explicitly cued fear responses compared with group housed mice (79; 71). The time-related increase of contextual fear responses correlated with the downregulation of 5α-reductase mRNA and protein expression observed in several corticolimbic areas, such as the frontal cortex, the hippocampus, and the amygdala (79).

Fear responses, similar to aggressive behavior, increased during the four weeks of social isolation to reach a plateau between four and eight weeks of isolation (79). Socially isolated mice also exhibited impaired and incomplete fear extinction (79). We had previously observed that socially isolated mice exhibited higher levels of anxiety-like behavior, determined by the elevated plus maze and in the open field (16; 40).

Several experiments have demonstrated that corticolimbic allopregnanolone plays a *pivotal* rather than incidental role in the regulation of contextual fear responses and aggression. First we showed that pharmacological treatment with allopregnanolone dose-dependently decreased aggression in a manner that correlated with an increase in corticolimbic allopregnanolone content (36). Allopregnanolone also normalized the exaggerated contextual fear responses and anxiety of socially isolated mice (79). Administration of the potent 5α-reductase competitive inhibitor SKF 105,111 to normal group-housed mice (43; 49; 71) rapidly (~1 h) decreased levels of allopregnanolone in the olfactory bulb, frontal cortex, hippocampus, and amygdala by 80–90% (71; 79) in association with a dose-dependent increase of conditioned contextual fear responses (79). The effects of SKF 105,111 on conditioned contextual fear responses were reversed by administering allopregnanolone doses that normalized hippocampus allopregnanolone levels (79). Our results are well in agreement with results of many other investigators who have observed that allopregnanolone elicits anxiolytic and antidepressant properties (40; 55; 82–88).

d. Altered $GABA_A$ receptors subunits

Increasing evidence originating from recent postmortem studies have provided support for the hypothesis that alterations in corticolimbic GABAergic neurotransmission, GABA receptor binding and receptor subunit composition, and GABA synthesis and transport are associated with several psychiatric disorders, including anxiety disorders, schizophrenia and depression (89–92).

The brain region-specific expression of $GABA_A$ receptor subunit subtypes has been associated with different physiological effects as well as the mediation of the pharmacological effects of $GABA_A$ receptor ligands that target specific subsets of $GABA_A$ receptor subunits (93; 94). For example, α1 -containing GABAA receptors have been thought to mediate the sedative properties of specific GABAergic ligands, while α2 and probably α3 subunits mediate anxiolysis, and α5 subunits appear to be involved in learning and cognition (93; 94). High affinity benzodiazepine binding to $GABA_A$ receptors requires the presence of an interface between α and γ subunits (93; 94).

In preclinical studies using socially isolated mice, we observed changes in the mRNA and protein expression of several $GABA_A$ receptor subunits in the frontal cortex and hippocampus (95). In particular, the mRNA levels encoding α1, α2, and γ2 GABAA receptor subunit subtypes were reduced by about 50%, while the mRNAs encoding α4 and α5 subunits were increased by about 130% compared to levels measured in group-housed mice (95). These results were confirmed in experiments in which α1 and α5 protein levels were assessed by Western blot in synaptic membrane preparations in the frontal cortex and hippocampus. We further studied whether the decline of $GABA_A$ receptor α1 subunit expression in the frontal cortex during social isolation is layer- or cell-specific (95). We used a laser microdissection technique coupled with nested RT-PCR amplification to determine the expression levels of α1 subunit mRNA in frontal cortex layer I, which is enriched in GABAergic interneurons and neuropil formed by the convergence of afferent thalamic fibers with apical dendrites of pyramidal neurons. We found that α1 mRNA levels were decreased by 50% in layer I neuropil, whereas the expression of α1 subunit mRNA in the pyramidal neurons of layer V was unchanged by social isolation. This demonstrated that the changes in $GABA_A$ receptor subunits within one brain area are region-specific rather than uniformly expressed (95).

These changes in $GABA_A$ receptor subunit subtype composition in socially isolated mice suggested that pharmacological responses to a range of $GABA_A$ receptor ligands might be altered. Interestingly, socially isolated mice showed a sustained resistance to the locomotor impairment (used as a measure of sedation) and anxiolytic action of diazepam and zolpidem, positive allosteric $GABA_A$ receptor modulators that bind with high affinity to α1, α2, α3 or α5 subunit-containing GABAA receptors (diazepam) and to α1 subunit-containing GABAA receptors (zolpidem) (95).

Previous studies have demonstrated that the α1 subunit of the GABAA receptor plays a primary role in mediating the sedative pharmacological effects of diazepam and zolpidem (93; 94; 96). Hence, the decreased responsiveness of socially isolated mice to the sedative effects of diazepam and zolpidem could be explained by a decrease in α1 subunit-containing GABAA receptors. Likewise, a decrease in the presence of γ2 subunits suggests the formation of GABAA receptors in which this subunit might be substituted. Given that γ2 subunits are a necessary prerequisite in the formation of benzodiazepine-sensitive $GABA_A$ receptors (93), our results suggests that the resistance of socially isolated mice to the anxiolytic activity of diazepam may result from the formation of benzodiazepine-insensitive $GABA_A$ receptors in neuronal circuits that play a pivotal role in regulating anxiety behaviors (40; 93; 95).

Social isolation-induced increases of α4 subunit-containing GABAA receptor expression in the frontal cortex appeared to be irrelevant to the behavioral or pharmacological alterations observed in socially isolated mice. In fact, GABA agonists such as THIP or the allosteric modulator, allopregnanolone, show selectivity and increased potency, respectively, for $GABA_A$ receptors containing α4/δ-subunits. At high doses, these compounds decrease locomotor activity comparably in group-housed and socially isolated mice (95). In contrast to diazepam, allopregnanolone dose-dependently induces a potent anxiolytic action in socially isolated mice (16; 40).

Several recent studies have suggested that expression of $GABA_A$ receptor subunits changes when brain neurosteroid levels decrease. For example, expression of α4–containing subunits increases during progesterone withdrawal or blockade of 5α–reductase (97). In socially isolated mice, allopregnanolone levels decrease in several corticolimbic structures that concomitantly show changes in $GABA_A$ receptor subunit mRNA and protein expression (79; 95). In future studies we plan to address whether social isolation directly affects the expression of $GABA_A$ receptor subunit composition or whether such changes are mediated via a decrease of corticolimbic neurosteroids, for example by decreasing the levels of 5α–DHP and its binding at nuclear progesterone receptors or by decreasing allopregnanolone.

SSRIs improve socially isolation-induced behavioral deficits acting as selective brain steroidogenic stimulants (SBSSs)

One of the best-characterized behavioral deficits induced by social isolation in rodents is the expression of aggressive behavior (98; 99). We observed that the expression of aggression is correlated with the extent of allopregnanolone level downregulation in several corticolimbic structures (36). Further, when the corticolimbic levels of allopregnanolone in socially isolated mice were normalized by a systemic administration with allopregnanolone, we observed a dose-dependent decrease of aggressive behavior to a same-sex intruder mouse (36). These antiaggressive effects of allopregnanolone were confirmed by experiment in which allopregnanolone was directly infused into the basolateral amygdala, which increased the levels of basolateral amygdala and hippocampus allopregnanolone levels (81). These results suggested that in socially isolated mice the decrease of corticolimbic allopregnanolone levels is responsible for the expression of aggression. However, our results contrast with investigations in which administration of allopregnanolone systemically induced a bitonic effect on aggressive behavior with low doses amplifying and larger doses decreasing the expression of aggression of mice (100; 101). It is however conceivable that experimental conditions, including housing conditions, in pairs (100) versus individual caging play a role in the observed behavioral differences of the response to allopregnanolone. As mentioned previously, individual caging is a procedure that results in a downregulation of allopregnanolone biosynthesis and administration of allopregnanolone in socially isolated mice results in a normalization of this neurosteroid's levels in the brain. Thus, a difference in allopregnanolone levels reached in crucial brain areas that regulate aggression may play a role in the discrepancy of the behavioral results observed in the two mouse models.

Several antidepressants of the SSRI type have been shown to potently increase the levels of allopregnanolone in rodents and depressed humans. Paroxetine and fluoxetine increased the levels of allopregnanolone in rats and mice without affecting the levels of pregnenolone or progesterone (35). Administration of a racemic mixture of the R- and S-isomers of fluoxetine induced increases in corticolimbic allopregnanolone levels and normalized the righting reflex loss induced by pentobarbital in mice (36–38). Importantly, at the doses used, fluoxetine failed to change the behavior and allopregnanolone levels of group housed mice (36; 37). In addition, inhibition of serotonin synthesis by treatment with p-

chlorophenylalanine failed to block the positive behavioral effects of fluoxetine, suggesting that the action of fluoxetine might be exerted by the ability of this drug to increase corticolimbic allopregnanolone levels (74).

Thus, we hypothesized that fluoxetine could ameliorate the behavioral deficits of socially isolated mice by upregulating corticolimbic allopregnanolone levels rather than by inhibiting serotonin reuptake. This hypothesis was investigated using the R- and S-stereoisomers of fluoxetine and norfluoxetine as pharmacological tools. We expected that these drugs would stereospecifically upregulate corticolimbic allopregnanolone content but have no stereoselectivity with regard to inhibition of 5-HT reuptake. In addition, we thought it possible that doses of fluoxetine and norfluoxetine stereoisomers that increase corticolimbic allopregnanolone content might differ from those that inhibit 5-HT reuptake. And indeed (16; 36–39; 71), fluoxetine dose-dependently and stereospecifically normalized the duration of pentobarbital-induced sedation and reduced aggressiveness, fear responses, and anxiety-like behavior at the same submicromolar doses that normalized the downregulation of brain allopregnanolone content in socially isolated mice. Interestingly, the S-stereoisomers of fluoxetine or norfluoxetine appeared to be 3 to 7 fold more potent than their respective R-stereoisomers and S-norfluoxetine was about 5-fold more potent than S-fluoxetine. Importantly, the effective concentrations (EC_{50}s) of S-fluoxetine and S-norfluoxetine that normalize the brain allopregnanolone content are 10- (S-fluoxetine) and 50-fold (S-norfluoxetine) lower than their respective EC_{50s} needed to inhibit 5-HT reuptake (36–39). Remarkably, the SSRI activity of S or R-fluoxetine and of S or R-norfluoxetine was devoid of stereospecificity (36; 37). *This study thus clearly demonstrated that neither the behavioral action nor the normalization of corticolimbic allopregnanolone content by S-fluoxetine and S-norfluoxetine is related to their intrinsic SSRI activity.*

The socially isolated mouse as a model for development of PTSD therapeutics

The findings that the socially isolated mouse expresses decreased levels of allopregnanolone biosynthesis, as well as changes in the expression of several $GABA_A$ receptor subunits in corticolimbic structures that regulate cognition and behaviors characteristic of anxiety and mood disorders such as PTSD and depression suggests that the *socially isolated mouse model* may be useful in investigating new molecules designed to improve behavioral deficits characterized by $GABA_A$ receptor signal transduction dysfunction (reviewed in 16; 39).

As in PTSD patients, the socially isolated mouse fails to respond to sedative and anxiolytic benzodiazepines. Our studies demonstrate that allopregnanolone administered systemically or directly infused into the basolateral amygdala has a strong anti-anxiety, anti-fear, and anti-aggression effect (36–39; 71; 81). These results were replicated by using S-norfluoxetine at doses that fail to have serotonergic effects but potently increase allopregnanolone biosynthesis in target corticolimbic areas, including the hippocampus, the basolateral amygdala, and the frontal cortex (81).

Allopregnanolone lacks $GABA_A$ receptor subunit selectivity and the functional $GABA_A$ receptor binding characteristics of benzodiazepines, which suggests that neurosteroids or molecules that stimulate neurosteroid biosynthesis might have an advantage over benzodiazepines in the treatment of psychiatric disorders in which neurosteroid downregulation and changes in $GABA_A$ receptor expression are operative. Allopregnanolone or fluoxetine and its congeners alleviate anxiety, fear, and aggressive behavior when benzodiazepines fail, due to their lack of $GABA_A$ receptor subtype selectivity. In addition, and in contrast to benzodiazepines, both allopregnanolone and SBSS

molecules decrease anxiety, fear, and aggression at concentrations that fail to impair locomotor activity (16; 36; 40; 79).

Thus, new SBSS molecules that fail to exert any significant SSRI activity should be explored for their ability to increase corticolimbic allopregnanolone levels and thereby ameliorate behavioral deficits in animal models of anxiety disorders and depression. Because allopregnanolone in similarity to short-acting benzodiazepines, such as alprazolam has a relatively short half-life and similar limiting pharmacokinetic properties, the best pharmacological approach consist of developing agents that stimulate neurosteroid synthesis rather than allopregnanolone analogs that directly substitute for allopregnanolone on their action on $GABA_A$ receptors. This approach is also of advantage because agents that stimulate allorepregnanolone synthesis allow to increase this neurosteroid's levels selectively in the brain areas that express neurosteroidogenic enzymes and where neurosteroidogenesis is deficient and, thus, is devoid of the global effects of administering neurosteroids or their analogs directly. Hence, agents that stimulate neurosteroidogenesis should be long-acting, such as norfluoxetine, so that they may continuously stimulate allopregnanolone neosynthesis and improve behavioral deficits regardless of allopregnanolone short-half life.

The high potency and stereospecificity of norfluoxetine in reducing behavioral deficits and in normalizing brain allopregnanolone content suggest that it may affect specific targets for regulating neurosteroidogenesis. The finding that protracted social isolation affects the expression of 5α-reductase in corticolimbic structures, but fails to change the expression of 3α-HSD, as well as the finding that brain progesterone levels do not change in socially isolated mice suggest that a mechanism involving 5α-reductase is responsible for the decrease of corticolimbic allopregnanolone content. This is further supported by the fact that 5α-reductase is the rate-limiting step-enzyme in allopregnanolone biosynthesis from progesterone (78). Furthermore, progesterone levels in group-housed and socially isolated mice are not affected by fluoxetine administration, suggesting that the SSRI/SBSSs impact neurosteroidogenesis downstream from progesterone (35; 74). The finding that low doses of the S isomers of fluoxetine or norfluoxetine increase corticolimbic levels of allopregnanolone in socially isolated mice, but fail to change levels in group-housed mice, suggests that 5α-reductase and/or 3α-HSD may become more susceptible to the effects of SBSSs during isolation (reviewed in 39). More investigations at the molecular enzymatic level are hence required to determine whether social isolation and neurosteroidogenic agents change the kinetics of 5α-reductase and/or 3α-HSD.

Effects of allopregnanolone and neurosteroidogenic antidepressants on neurogenesis and neuronal survival

Neurogenesis in the hippocampus is susceptible to internal and external factors. Social stress, including protracted social isolation decreases it (102). Accordingly, rodent models of stress-induced depression show impaired neurogenesis (103, 104). Interestingly, antidepressant treatments, environmental enrichment, exercise, and learning are able to increase the number, differentiation and survival of newborn hippocampal neurons (105–108). The pharmacological effects of neurosteroidogenic antidepressants can be abolished by reducing dentate gyrus neurogenesis (109–111), suggesting that the pharmacological effects of antidepressants may include the stimulation of neural progenitor cells, as reported in studies with rodents, humans, and nonhuman primates (106, 112). The basolateral amygdala also seems to play a role in antidepressant-mediated hippocampal cell proliferation and survival. Fluoxetine was shown to have a positive effect on hippocampal cell survival and an antidepressant action in rodents only when the basolateral amygdala was lesioned (113).

Gluococorticoids exert a primary and permissive regulatory role in hippocampal neurogenesis; stress-induced increases in glucocorticoid levels reduce the proliferation of progenitor cells in the dentate gyrus; in contrast, a reduction in glucocorticoid levels induced by adrenalectomy enhances neurogenesis (114). DHEA can also regulate neurogenesis. DHEA given to rats stimulated progenitor cell division and counteracted the suppressive effects of corticosterone (115). Interestingly, DHEA showed a synergistic effect with antidepressants. DHEA added to an otherwise ineffective dose of fluoxetine increased progenitor cell proliferation to the same extent as doses four time higher, supporting a role for DHEA as a useful adjunct therapy for depression (116).

The participation of progesterone and progesterone metabolites including allopregnanolone in neurogenesis and neuronal survival has been investigated in several recent studies.

In the hippocampal dentate gyrus of adult male mice, administration of progesterone increased the number of cells by two-fold, likely by enhancing survival of newborn neurons (117). The effects of progesterone appeared to be partially mediated by binding to progesterone receptors as suggested by the fact that the progesterone antagonist RU486 partially blocked it, while the 5α-reductase inhibitor finansteride failed to prevent this effect (117).

In an in vitro study, both progesterone and allopregnanolone promoted human and rat neural progenitor cell proliferation with allopregnanolone showing the greater efficacy at the same concentration (118). In *in vivo* studies, allopregnanolone increased BrdU incorporation into 3-month-old mouse hippocampal subgranular zone (SGZ) as well as the subventricular zone (SVZ), (119, 120). These data suggested that for both neuroprotection and likely for neurogenesis, allopregnanolone is the preliminary active agent. The role of allopregnanolone on neurogenesis was also tested on cerebellar granule cells. Allopregnanolone increased proliferation of immature cerebellar granular cells taken from 6-8-day-old pups. This effect was abolished by bicuculline, picrotoxin, and nifedipine, suggesting that allopregnanolone increases DNA synthesis through a $_{GABAA}$ receptor-mediated activation (121).

The normal aged-related decline of allopregnanolone or its and other neurosteroid decline in psychiatric or neurological disorders (e.g., Alzheimer's Disease) may trigger the subsequent decrease of neurogenesis and decreased expression of growth factors (110). Hence, restored brain content of allopregnanolone following treatment with a steroidogenic drug reverses neurogenesis downregulation and improves emotional and cognitive functions (106, 110, 122).

Relationship between brain derived neurotrophic factor (BDNF) and allopregnanolone in corticolimbic neurons

BDNF is synthesized in a pro- and mature- form and can be released from neurons by either a constitutive secretion or activity-dependent release (reviewed in 123). Upon its release, BDNF binds to two different receptors, the tropomiosin-related kinase receptor type B (TRKB), and the p75 receptor (123). BDNF plays a pivotal role in the brain plasticity associated with learning and memory. In the adult brain, it supports dendritic spine morphology and branching, as well as synaptic plasticity and long-term potentiation (LTP), thus maintaining correct tropism (123, 124).

Plasticity in corticolimbic circuits is a prerequisite for the triggering extinction of fear conditioned responses (125, 126). In these circuits, BDNF mediates plasticity and can be epigenetically regulated in a manner that correlates with fear extinction (127). Moreover, a decrease of BDNF has been implicated in the mechanisms underlying the clinical

manifestations of PTSD and in the impairment of cognitive function in psychiatric disorders. Interestingly, both PTSD and depressed patients express decreased levels of BDNF in the hippocampus and plasma (128). Of note, PTSD and depression have consistently been associated with decreased hippocampal volume with no differences in total cerebral volume and with functional impairments (129–132). The hippocampal volume loss appears to have functional significance as it is associated with memory loss (123). These studies collectively suggest that depression and PTSD are associated with hippocampal atrophy. Therapy using the neurosteroidogenic antidepressants resulted in an improvement of PTSD and depressive symptoms and in a significant improvement in mean hippocampal volume (123, 133). These neurosteroidogenic antidepressants have been reported in several studies to upregulate serum and hippocampal BDNF levels, which correlated with improved symptoms (128, 134, 135), suggesting that antidepressant-mediated BDNF upregulation may counteract the hippocampal atrophy by stimulating dendritic spine arborization and morphology and neurogenesis.

Based on these reports and on clinical and preclinical studies from our own group, we hypothesize that in PTSD and depressed patients who show a decrease of CSF allopregnanolone levels, antidepressant-induced allopregnanolone level upregulation may exert an antidepressant effect by elevating BDNF levels. This hypothesis is supported in part by studies conducted in our laboratory. A decrease of corticolimbic allopregnanolone levels in socially isolated mice is associated with decreased levels of corticolimbic BDNF mRNA expression in the same brain areas, namely medial frontal cortex, hippocampus, and BLA (79, 136). These neurochemical deficits are associated with behavioral dysfunction, including exaggerated contextual fear conditioning and impaired extinction, anxiety-like behavior, and aggressiveness (136). Of note, in socially isolated mice we further studied mean spine density and the percentage of mature spines in layer III of the frontal cortex. Spine density was lower for socially isolated mice along the entire extent of the apical and basilar dendrites. Socially isolated mice also had a lower percentage of mature spines on both the apical and basilar dendrites. For the apical dendrite, the greatest decrease in mature spines was in the proximal portion of the dendrite, while for the basilar dendrites, the greatest decrease in mature spines was in the mid and distal portion of the dendrite (137, and manuscript in preparation).

Also, allopregnanolone treatment or S-norfluoxetine, at concentrations sufficient to increase corticolimbic allopregnanolone levels, also normalized corticolimbic BDNF mRNA expression and improved dendritic spine morphology as well as behavioral deficits in socially isolated mice (136). S-norfluoxetine and allopregnanolone treatment induced a reduction of conditioned fear, facilitated fear extinction, and prevented the reinstatement of fear memory following extinction (79, 136). Finally, and importantly, S-norfluoxetine and allopregnanolone actions on conditioned fear responses were mimicked by a single bilateral microinfusion of BDNF into the BLA, which dose-dependently facilitated fear extinction and abolished the reinstatement of fear responses in the absence of locomotion impairment (138). These observations thus support the hypothesis that by increasing allopregnanolone levels, SBSS drugs such as S-norfluoxetine may be involved in the regulation of corticolimbic BDNF expression and may induce a long-term improvement in the behavioral dysfunctions related to PTSD. Further work will be needed to determine definitively whether there is a direct mechanistic, rather than epiphenomenal connection between the observed changes in allopregnanolone and BDNF, however.

New therapeutic strategies for PTSD: Integration of clinical and preclinical leads

A variety of clinical studies have implicated alterations in GABAergic neurotransmission in the pathophysiology of depression and PTSD. Decreased peripheral and central nervous system GABA levels, reductions in $GABA_A$ and $GABA_B$ receptor binding and/or sensitivity, as well as reduced functional measures of GABAergic tone have been found in a variety of depressed patient populations, most consistently and profoundly among treatment resistant patients (139, 140). In PTSD, decreased frontal lobe benzodiazepine receptor binding (141, 142) and decreased plasma GABA levels (143) have been demonstrated, while a polymorphism in the gene for the $GABA_A$ receptor β3 subunit was associated with higher levels of somatic symptoms, depression, anxiety, and insomnia in PTSD (144). Increased plasma levels of dehydroepiandrosterone (DHEA) and/or its sulfated metabolite (DHEAS), androgenic steroids with negative modulatory effects at brain $GABA_A$ receptors, have been seen in combat veterans with PTSD (145) and refugees from Kosovo who developed PTSD with greater sleep disturbance (146), while premenopausal women with chronic PTSD have shown greater DHEA release after maximal adrenal stimulation by adrenocorticotropic hormone ($ACTH_{1–24}$) (147).

Interestingly, then, benzodiazepines have not been found to effectively treat PTSD (10, 11, 12). In addition, while the SSRIs sertraline and paroxetine are the only medications currently approved by the Federal Drug Administration (FDA) for the treatment of PTSD, their effect sizes are modest (148–151), and in some PTSD populations, including American male combat veterans, they were found to be ineffective (152).

Interestingly, a critical role for benzodiazepine-resistant extrasynaptic $GABA_A$ receptors, but not benzodiazepine-sensitive synaptic $GABA_A$ receptors, during recovery from traumatic stress exposure is indirectly supported by preclinical research. Tonic, slowly desensitizing GABA-mediated inhibitory currents are maintained by extrasynaptic $GABA_A$ receptors, while benzodiazepine-sensitive $GABA_A$ receptors rapidly desensitize in the face of high or persistent GABA exposure (153), as would occur during prolonged stress or fear conditioning. In addition, Ressler et al (154) showed that gephyrin (a synaptic protein that clusters $GABA_A$ receptors at neuronal synapses), gephyrin mRNA, and benzodiazepine-sensitive synaptic $GABA_A$ receptors in the amygdala are down-regulated as a result of fear conditioning, while levels of gephyrin and synaptic $GABA_A$ receptors are restored following extinction training (155). This could account for the ineffectiveness of benzodiazepines in facilitating extinction and in treating PTSD. This also suggests that potentiation of extra-synaptic $GABA_A$ receptor function by stress-related surges in allopregnanolone (156) during re-exposure to trauma-related cues may provide a critical level of inhibitory tone, restraining amygdala-mediated effects on arousal and defensive responding and thereby facilitating higher order processing and extinction of conditioned negative emotions and defensive behaviors. Failure to sufficiently activate these receptors in the aftermath of trauma thus may potentiate the risk for development of chronic PTSD.

As discussed earlier, our clinical research suggests that there may be a block in synthesis of allopregnanolone in some individuals with PTSD and depression, with allopregnanolone levels appearing to be lowest among patients comorbid for both disorders (17). Of note, DSMIV-defined PTSD and major depressive disorder (MDD) are frequently comorbid and share multiple symptoms. Indeed, several epidemiological studies show that depression diagnosed after trauma exposure is almost always comorbid with PTSD, leading some investigators to suggest that comorbid PTSD/MDD may be essentially more severe PTSD (157).

There are several mechanisms by which conversion of the progesterone derivative 5α-DHP to allopregnanolone could be deficient in PTSD and/or depression. The enzyme that converts 5α-DHP to allopregnanolone, 3α-HSD, operates in a bidirectional manner with the direction of its effects dependent on the oxidative state of its cofactor, nicotinamide adenine dinucleotide phosphate ($NADP^+$ vs. NADPH). It is thus possible that faulty inhibition of 3α-HSD oxidase activity by accumulated NADPH under conditions of stress or deficient accumulation of NADPH—for example, due to stimulation of NADPH oxidase--could result in preferential back-conversion of allopregnanolone to 5α-DHP and deficient allopregnanolone production during stress (17). Interestingly, ethanol stimulates NADPH oxidase (e.g., 158) and administration of chronic intermittent ethanol to rats has been shown to produce a decrease in brain allopregnanolone relative to 5α–DHP levels (159)—a pattern similar to that seen in the cerebrospinal fluid of women with PTSD (17). Given that alcohol dependence is another frequent comorbid condition in PTSD, it is possible that deficits in allopregnanolone activity at the alcohol sensitive, benzodiazepine resistant extrasynsaptic $GABA_A$ receptors may contribute significantly to the pathophysiology of both disorders.

In addition to dysfunction of the enzyme involved in allopregnanolone synthesis, a functional polymorphism or frank mutation of the 3α-HSD gene or other cause for dysregulation of 3α-HSD gene expression could lead to reduced CSF allopregnanolone levels (160). The human 3α-HSD gene is located between 10p15 and 10p14 on chromosome 10. The human gene is similar to the rat 3α-HSD gene, in that it has nine exon-intron boundaries and codes for four 3α-HSD isoforms with differing tissue distributions and ligand affinities (160). These isoforms share 86% sequence identity and include: AKR1C1 (20α) 3α-HSD, AKR1C2 (type 3 3α–HSD or bile-acid binding protein found in prostate and mammary gland); AKR1C3 (type 2 3α-HSD or type 5 17β-HSD); and AKR1C4 (type1 3α-HSD virtually found only in liver). The type 2 and type 3 isoforms are known to reduce potent testosterone (5α-DHT) to the weak androgen 3a-diol and thus regulate occupancy of androgen receptors (160). The type 3 isoform is present in the CNS and preferentially converts 5α-DHP to allopregnanolone. In general, the AKR1C isoforms are plastic and able to work as either 3-, 17- or 20-ketosteroid reductases; in addition, because they interconvert active and cognate inactive steroids, they contribute to control of the occupancy of estrogen, progesterone, and testosterone, as well as GABA receptors.

The promoter region of the 3α-HSD gene (161) contains an AP-1 binding site, as well as a negative response element (NRE) that enables constitutive *repression* of the gene by the transcription factor, OCT-1. A functional steroid response unit (SRU) in the promoter enables *upregulation* of the gene by progesterone, estrogen, and glucocorticoids. Tomkins (162) first demonstrated a negative feedback role for the 3α-HSD gene in regulation of steroid levels: Rising glucocorticoid levels increased 3α-HSD gene transcription and subsequent metabolism of glucocorticoids in the liver. Further work by Hou et al. (163) revealed that the occupied glucocorticoid Type 2 receptor binds to a cis-glucocorticoid response element (GRE) in the promoter of the 3α-HSD gene to increase liver (Type I) 3α-HSD gene transcription.

The capacity to correct allopregnanolone deficits in patients with PTSD or depression thus may depend on individually diverse mechanisms by which allopregnanolone synthesis is impaired. While SSRIs clearly increase allopregnanolone levels (at least in healthy animals and perhaps even in most patients with isolated major depression in whom they are therapeutic), the precise mechanism by which SSRIs increase allopregnanolone is unclear. As previously noted, work by Mellon et al. (164) suggested that SSRIs directly stimulate activity of 3α-HSD, though subsequent work by Trauger et al. (165) failed to support this mechanism. More recent work suggests that fluoxetine inhibits NADPH oxidase (166), which in turn could increase NADPH levels and shift the action of 3α-HSD towards 5α-

DHP reduction and allopregnanolone production. In individuals for whom administration of an SSRI (or SBSS) is ineffective, administration of an allopregnanolone substitute (e.g. 167, 168), such as a 3β-methylated synthetic analog of allopregnanolone, ganaxolone (3α-hydroxy-3β-methyl-5α-pregnan-20-one), may be therapeutic. Indeed, a multisite Phase II clinical trial of the efficacy and safety of ganaxolone in patients with PTSD is currently underway. Alternatively, medications such as topiramate, which act in part by direct activation of extrasynaptic $GABA_A$ receptors (169, 170), may benefit such individuals.

Medications that increase plasma allopregnanolone levels by other mechanisms (e.g., DHEA, olanzepine, or even clozapine) also may be effective on this basis in some individuals with PTSD (171–176). Administration of glucocorticoids, which have demonstrated some preliminary benefit in preventing or treating PTSD (e.g. 177), may not only correct potential cortisol synthesis deficits but also increase allopregnanolone production in some patients with PTSD. Novel agents, such as NADPH oxidase inhibitors, also could potentially be used to invigorate reductive metabolism by 3α-HSD during stress. Currently available medications with NADPH oxidase inhibition as part of a their pharmacological profile are currently used to treat PTSD (e.g., fluoxetine), as well as medical disorders such as cardiovascular disease (178), which is frequently comorbid with PTSD and thought to have a related pathophysiological basis (179). Novel and perhaps more selective NADPH oxidase inhibitors, which have only undergone trials in animals (e.g. apocynin), or variations thereof, could potentially be developed for use in humans to invigorate reductive metabolism during stress or stress recovery to increase allopregnanolone and reduce the production of harmful radical oxygen species.

The translocase protein (18 kDa) or TSPO, previously called mitochondrial peripheral benzodiazepine receptor or PBR, is another possible pharmacological target to enhance corticolimbic allopregnanolone biosynthesis (30, 31, 80). TSPO represents the starting point and an important rate-limiting step in neurosteroidogenesis. It “gates” the availability of neurosteroids in the brain by regulating their production from cholesterol (80). Neurosteroidogenesis starts with the transport of cholesterol into inner mitochondrial membranes and its conversion to pregnenolone by P450scc, which is located in the inner mitochondrial membrane (80). A cascade of enzymatic processes then take place in the cytosol, resulting in the production of neuroactive steroids, including pregnenolone sulfate, DHEAS [(though apparently not in human brain (180)], THDOC, and allopregnanolone (reviewed in 32). Thus, new molecules that bind with high affinity to TSPO have been recently investigated. These drugs are able to exert important anxiolytic effects but are devoid of the unwanted side effects associated with benzodiazepines, including over-sedation and tolerance (30; 31, 80). In mouse models, TSPO agents have been shown to potently increase pregnenolone levels in corticolimbic structures, including hippocampus and cortex, as well as to induce anxiolytic effects (181–184). TSPO ligands include XBD173 and etifoxine, which have proven to be highly efficacious anxiolytic and antidepressant drugs in a number of behavioral tests (185). The anxiolytic/antidepressant effects of these agents were related their ability to increase neurosteroid biosynthesis, as confirmed by studies in which key enzyme blockers for neurosteroid biosynthesis, including finansteride and trilostane (31, 57), were used. Indeed, TSPO ligands have recently showed promising therapeutic effects in clinical studies (30, 31).

BDNF is also a highly promising target for the development of PTSD therapies (186). The challenge in using BDNF, however, has been the difficulty of delivering BDNF to the brain (123). Thus, molecules that are able to stimulate BDNF levels, including the SBSSs discussed above, may bypass the delivery challenge represented by administering BDNF directly.

Importantly all of the potentially therapeutic agents discussed above will need to be carefully tested for safety as well as efficacy in appropriately powered clinical studies in PTSD.

Conclusion

The use of a new class of pharmacological tools, the SBSSs (selective brain steroidogenic stimulants) has emerged as a new therapeutic strategy for the treatment of psychiatric disorders associated with a downregulation of brain allopregnanolone biosynthesis, including anxiety disorders, depression, and PTSD. As discussed, the SBSSs appear to be more efficacious than benzodiazepines, as well as devoid of the unwanted side-effects induced by benzodiazepines.

The pharmacological spectrum of allopregnanolone as an allosteric modulator of GABA at $GABA_A$ receptors is broader than that of benzodiazepines, which fail to modulate $GABA_A$ receptors containing α4 and α6 subunits. The efficacy of allopregnanolone in positively modulating GABA action at $GABA_A$ receptors is expressed widely in several subtypes of $GABA_A$ receptors at doses in the low nM range. Hence, selective stimulation of allopregnanolone biosynthesis may avoid the therapeutic hindrances caused by the formation of benzodiazepine-resistant $GABA_A$ receptors with altered subunit composition, such as may occur in stress-related psychiatric disorders (reviewed in 187).

The molecular mechanisms subserving the SBSS-induced facilitation of neurosteroidogenesis remain to be elucidated. However, given the brain region- and neuron-specific expression of 5α-reductase and 3α-HSD, the action of SBSSs on neurosteroid biosynthesis is likely to be neuron and site-specific. Hence, SBSSs such as S-norfluoxetine and its congeners, that specifically target key enzymes downstream in allopregnanolone neosynthesis, may be devoid of unwanted side effects induced by activation of a broader neurosteroidogenic cascade within a broader array of brain targets.

In summary, novel SBSS drugs that specifically increase corticolimbic allopregnanolone biosynthesis appear to be a promising new pharmacological class of future drugs for the treatment of anxiety disorders and PTSD.

Acknowledgments

This review was supported by the National Institute of Mental Health Grant MH 085999 (to G. Pinna) and VA Boston Healthcare System and National Institute of Mental Health Grant MH31113 (to A. Rasmusson).

Reference List

1. Diagnostic Statistical Manual of Mental Disorders. Washington DC: American Psychiatric Press; 2000.
2. Berton O, Nestler IJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 2006; 7:137–151. [PubMed: 16429123]
3. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry. 2005; 62:593–602. [PubMed: 15939837]
4. Costa E, Guidotti A. Benzodiazepines on trial: a research strategy for their rehabilitation. Trends Pharmacol Sci. 1996; 17:192–200. [PubMed: 8669126]
5. Bandelow B, Zohar J, Hollander E, Kasper S, Möller HJ, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and post-traumatic stress disorders - first revision. World J Biol Psychiatry. 2008; 9:248–312. [PubMed: 18949648]

6. Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, Davidson JR, den Boer JA, Fineberg NA, Knapp M, Scott J, Wittchen HU. British Association for Psychopharmacology. Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. J Psychopharmacol. 2005; 19:567–96. [PubMed: 16272179]
7. Rudolph U, Möhler H. GABA-based therapeutic approaches: $GABA_A$ receptor subtype functions. Curr Opin Pharmacol. 2006; 6:18–23. [PubMed: 16376150]
8. Nutt DJ, Ballenger JC, Sheehan D, Wittchen HU. Generalized anxiety disorder: comorbidity, comparative biology and treatment. Int J Neuropsychopharmacol. 2002; 5:315–25. [PubMed: 12466031]
9. Pinna G, Galici R, Schneider HH, Stephens DN, Turski L. Alprazolam dependence prevented by substituting with the beta-carboline abecarnil. Proc Natl Acad Sci USA. 1997; 94:2719–23. [PubMed: 9122263]
10. Viola J, Ditzler T, Batzer W, Harazin J, Adams D, Lettich L, Berigan T. Pharmacological management of post-traumatic stress disorder: clinical summary of a five-year retrospective study, 1990–1995. Mil Med. 1997; 162:616–9. [PubMed: 9290298]
11. Davidson JR. Use of benzodiazepines in social anxiety disorder, generalized anxiety disorder, and posttraumatic stress disorder. J Clin Psychiatry. 2004; 65:29–33. [PubMed: 15078116]
12. Gelpin E, Bonne O, Peri T, Brandes D, Shalev AY. Treatment of recent trauma survivors with benzodiazepines: a prospective study. J Clin Psychiatry. 1996; 57:390–4. [PubMed: 9746445]
13. Atack JR. Anxioselective compounds acting at the GABA(A) receptor benzodiazepine binding site. Curr Drug Targets CNS Neurol Disord. 2003; 2:213–32. [PubMed: 12871032]
14. Costa E. From $_{GABAA}$ receptor diversity emerges a unified vision of GABAergic inhibition. Annu Rev Pharmacol Toxicol. 1998; 38:321–50. [PubMed: 9597158]
15. Puia G. Molecular mechanisms of the partial allosteric modulatory effects of bretazenil at gamma-aminobutyric acid type A receptor. Proc Natl Acad Sci USA. 1992; 89:3620–4. [PubMed: 1373505]
16. Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically and selectively increase brain neurosteroid content at doses that are inactive on 5-HT reuptake. Psychopharmacology. 2006; 186:362–372. [PubMed: 16432684]
17. Rasmusson AM, Pinna G, Paliwal P, Weisman D, Gottschalk C, Charney D, et al. Decreased cerebrospinal fluid allopregnanolone levels in women with posttraumatic stress disorder. Biol Psychiatry. 2006; 60:704–13. [PubMed: 16934764]
18. Nappi RE, Petraglia F, Luisi S, Polatti F, Farina C, Genazzani AR. Serum allopregnanolone in women with postpartum “blues”. Obstet Gynecol. 2001; 97:77–80. [PubMed: 11152912]
19. Ströhle A, Romeo E, di Michele F, Pasini A, Yassouridis A, Holsboer F, Rupprecht R. $GABA_A$ receptor-modulationg neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment. Am J Psychiatry. 2002; 159:145–7. [PubMed: 11772707]
20. Rapkin AJ, Morgan M, Goldman L, Brann DW, Simone D, Mahesh VB. Progesterone metabolite allopregnanolone in women with presmenstrual syndrome. Obstet Gynecol. 1997; 90:709–14. [PubMed: 9351749]
21. Marx CE, Keefe RS, Buchanan RW, Hamer RM, Kilts JD, Bradford DW, et al. Proof-of concept trial with the neurosteroid pregnenolone targeting cognitive and negative symptoms in schizophrenia. Neuropsycopharmacology. 2009; 34:1885–903.
22. Pearlstein T. Premenstrual dysphoric disorder: out of the appendix. Arch Womens Ment Health. 2010; 13:21–3. [PubMed: 20127448]
23. Amin Z, Mason GF, Cavus I, Krystal JH, Rothman DL, Epperson CN. The interaction of neuroactive steroids and GABA in the development of neuropsychiatric disorders in women. J Psychopharmacol. 2007; 21:414–20. [PubMed: 16891341]
24. Bäckström T, Andreen L, Birzniece V, Björn I, et al. The role of hormones and hormonal treatments in premenstrual syndrome. CNS Drugs. 2003; 17:325–42. [PubMed: 12665391]
25. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of gonadal steroids in women with a history of postpartum depression. Am J Psychiatry. 2000; 157:924–30. [PubMed: 10831472]

26. Uzunova V, Sheline Y, Davis JM, Rasmusson A, Uzunov DP, Costa E, Guidotti A. Increase in the cerebrospinal fluid content of neurosteroids in patients with unipolar major depression who are receiving fluoxetine or fluvoxamine. Proc Natl Acad Sci USA. 1998; 95:3239–44. [PubMed: 9501247]
27. Romeo E, Ströhle A, Spalletta G, di Michele F, Hermann B, Holsboer F, Pasini A, Rupprecht R. Effects of antidepressant treatment on neuroactive steroids in major depression. Am J Psychiatry. 1998; 155:910–3. [PubMed: 9659856]
28. Serra M, Madau P, Chessa MF, Caddeo M, Sanna E, Trapani G, Franco M, Liso G, Purdy RH, Barbaccia ML, Biggio G. 2-Phenyl-imidazo[1,2-a]pyridine derivatives as ligands for peripheral benzodiazepine receptors: stimulation of neurosteroid synthesis and anticonflict action in rats. Br J Pharmacol. 1999; 127:177–87. [PubMed: 10369471]
29. Rupprecht R, Rammes G, Eser D, Baghai TC, et al. Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. Science. 2009; 325:490–3. [PubMed: 19541954]
30. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D, Schumacher M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Rev Drug Discov. 2010; 9:971–88. [PubMed: 21119734]
31. 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 [Epub ahead of print].
32. Costa B, Da Pozzo E, Chelli B, Simola N, Morelli M, Luisi M, Maccheroni M, Taliani S, Simorini F, Da Settimo F, Martini C. Anxiolytic properties of a 2-phenylindolglyoxylamide TSPO ligand: Stimulation of in vitro neurosteroid production affecting $GABA_A$ receptor activity. Psychoneuroendocrinology. 2011; 36:463–472. [PubMed: 20728278]
33. Agis-Balboa, RC.; Guidotti, A.; Whitfield, H.; Pinna, G. 2010 Neuroscience Meeting. San Diego, CA: Society for Neuroscience; 2010. Allopregnanolone biosynthesis is downregulated in the prefrontal cortex/Brodmann's area 9 (BA9) of depressed patients.
34. van Broekhoven F, Verkes RJ. Neurosteroids in depression: a review. Psychopharmacology. 2003; 165:97–110. [PubMed: 12420152]
35. Uzunov DP, Cooper TB, Costa E, Guidotti A. Fluoxetine elicited changes in brain neurosteroid content measured by negative ion mass fragmentography. Proc Natl Acad Sci USA. 1996; 93:12599–12604. [PubMed: 8901628]
36. Pinna G, Dong E, Matsumoto K, Costa E, Guidotti A. In socially isolated mice, the reversal of brain allopregnanolone down-regulation mediates the anti-aggressive action of fluoxetine. Proc Natl Acad Sci USA. 2003; 100:2035–2040. [PubMed: 12571361]
37. Pinna G, Costa E, Guidotti A. Fluoxetine and norfluoxetine stereospecifically facilitate pentobarbital sedation by increasing neurosteroids. Proc Natl Acad Sci USA. 2004; 101:6222–6225. [PubMed: 15069199]
38. Pinna G, Costa E, Guidotti A. SSRIs act as selective brain steroidogenic stimulants (SBSSs) at low doses that are inactive on 5-HT reuptake. Curr Opin Pharmacol. 2009; 9:24–30. [PubMed: 19157982]
39. Pinna G. In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis. Behav Pharmacol. 2010; 21:438–50. [PubMed: 20716970]
40. Nin, Schuler M.; Martinez, LA.; Thomas, R.; Nelson, M.; Pinna, G. Allopregnanolone and S-norfluoxetine decrease anxiety-like behavior in a mouse model of anxiety/depression. Trabajos del Instituto Cajal. 2011; 83:215–216.
41. Baulieu, EE. Steroid hormones in the brain: several mechanisms. In: Fuxe, K.; Gustafson, JA.; Wettenberg, L., editors. Steroid hormone regulation of the brain. Elmsford, NY: Pergamon; 1981. p. 3-14.
42. Baulieu EE, Robel P, Schumacher M. Neurosteroids: beginning of the story. Int Rev Neurobiol. 2001; 46:1–32. [PubMed: 11599297]
43. Cheney DL, Uzunov D, Costa E, Guidotti A. Gas chromatographic-mass fragmentographic quantitation of 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and its precursors in blood and

brain of adrenalectomized and castrated rats. J Neurosci. 1995; 15:4641–4650. [PubMed: 7790929]

44. Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders. Brain Res Rev. 2001; 37:110–115. [PubMed: 11744079]
45. Stoffel-Wagner B. Neurosteroid metabolism in the human brain. European Journal of Endocrinology. 2001; 145:669–679. [PubMed: 11720889]
46. Puia G, Santi MR, Vicini S, Pritchett DB, Purdy RH, Paul SM, Seeburg PH, Costa E. Neurosteroids act on recombinant human $GABA_A$ receptors. Neuron. 1990; 4:759–765. [PubMed: 2160838]
47. Puia G, Vicini S, Seeburg PH, Costa E. Influence of recombinant gamma-aminobutyric acid—a receptor subunit composition on the action of allosteric modulators of gammaaminobutyricacid-gated Cl-currents. Mol Pharmacol. 1991; 39:691–696. [PubMed: 1646944]
48. Puia G, Mienville J-M, Matsumoto K, Takahata H, Watanabe H, Costa E, Guidotti A. On the putative physiological role of allopregnanolone on $GABA_A$ receptor function. Neuropharmacology. 2003; 44:49–55. [PubMed: 12559121]
49. Pinna G, Uzunova V, Matsumoto K, Puia G, Mienville J-M, Costa E, Guidotti A. Brain allopregnanolone regulates the potency of the $GABA_A$ receptor agonist muscimol. Neuropharmacology. 2000; 39:440–448. [PubMed: 10698010]
50. Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA. Neurosteroid modulation of $GABA_A$ receptors. Prog Neurobiol. 2003; 71:67–80. [PubMed: 14611869]
51. Lambert JJ, Cooper MA, Simmons RD, Weir CJ, Belelli D. Neurosteroids: endogenous allosteric modulators of $GABA_A$ receptors. Psychoneuroendocrinology. 2009; 34 (Suppl 1):S48–58. [PubMed: 19758761]
52. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nat Rev Neurosci. 2005; 6:565–575. [PubMed: 15959466]
53. Majewska MD. Neurosteroids: endogenous bimodal modulators of the $GABA_A$ eceptor. Mechanism of action and physiological significance. Prog Neurobiol. 1992; 38:379–395. [PubMed: 1349441]
54. D'Aquila PS, Canu S, Sardella M, Spanu C, Serra G, Franconi F. Dopamine is involved in the antidepressant-like effect of allopregnanolone in the forced swimming test in female rats. Behav Pharmacol. 2010; 21:21–8. [PubMed: 20009921]
55. Nin MS, Salles FB, Azeredo LA, Frazon AP, Gomez R, Barros HM. Antidepressant effect and changes of $GABA_A$ receptor gamma2 subunit mRNA after hippocampal administration of allopregnanolone in rats. J Psychopharmacol. 2008; 22:477–85. [PubMed: 18308780]
56. Rodríguez-Landa JF, Contreras CM, García-Ríos RI. Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the $GABA_A$ receptor. Behav Pharmacol. 2009; 20:614–622. [PubMed: 19752723]
57. Kita, Furukawa. Involvement of neurosteroids in the anxiolytic-like effects of AC-5216 in mice. Pharmacol Biochem Behav. 2008; 89:171–8. [PubMed: 18201755]
58. Lonsdale, Burnham. The anticonvulsant effects of allopregnanolone against amygdalakindled seizures in female rats. Neurosci Lett. 2007; 411:147–51. [PubMed: 17084970]
59. Mares P, Mikulecká A, Haugvicová R, Kasal A. Anticonvulsant action of allopregnanolone in immature rats. Epilepsy Res. 2006; 70:110–7. [PubMed: 16644184]
60. Martin-Garcia E, Pallares M. The intrahippocampal administration of the neurosteroid allopregnanolone blocks the audiogenic seizures induced by nicotine. Brain Res. 2005; 1062:144–50. [PubMed: 16256958]
61. Jain NS, Hirani K, Chopde CT. Reversal of caffeine-induced anxiety by neurosteroid 3- alpha-hydroxy-5-alpha-pregnane-20-one in rats. Neuropharmacology. 2005; 48:627–38. [PubMed: 15814098]
62. Guidotti A, Dong E, Matsumoto K, Pinna G, Rasmusson AM, Costa E. The socially-isolated mouse: a model to study the putative role of allopregnanolone and 5α-dihydroprogesterone in psychiatric disorders. Brain Res Rev. 2001; 37:110–115. [PubMed: 11744079]

63. Puia G, Ducici I, Vicini S, Costa E. Does neurosteroid modulatory efficacy depend on $GABA_A$ receptor subunit composition? Receptors Channels. 1993; 1:135–42. [PubMed: 8081717]
64. Matsumoto K, Nomura H, Murakami Y, Taki K, Takahata H, Watanabe H. Long-term social isolation enhances picrotoxin seizure susceptibility in mice: up-regulatory role of endogenous brain allopregnanolone in GABAergic systems. Pharm Biochem Behav. 2003; 75:831–835.
65. Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites. Nature. 2006; 444:486–9. [PubMed: 17108970]
66. Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with $GABA_A$ receptors. Neuropharmacology. 2002; 43:651–61. [PubMed: 12367610]
67. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the $GABA_A$ receptor. Nat Rev Neurosci. 2005; 6:565–575. [PubMed: 15959466]
68. Vicini S. Pharmacologic significance of the structural heterogeneity of the $GABA_A$ receptor-chloride ion channel complex. Neuropsychopharmacology. 1991; 4:9–15. [PubMed: 1706183]
69. Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. Proc Natl Acad Sci USA. 2006; 103:14602–14607. [PubMed: 16984997]
70. Agis-Balboa RC, Pinna G, Kadriu B, Costa E, Guidotti A. Downregulation of 5α-reductase type I mRNA expression in cortico-limbic glutamatergic circuits of mice socially isolated for four weeks. Proc Natl Acad Sci USA. 2007; 104:18736–41. [PubMed: 18003893]
71. Pinna G, Agis-Balboa R, Pibiri F, Nelson M, Guidotti A, Costa E. Neurosteroid biosynthesis regulates sexually dimorphic fear and aggressive behavior in mice. Neurochemical Research. 2008; 33:1990–2007. [PubMed: 18473173]
72. Akk G, Shu HJ, Wang C, Steinbach JH, Zorumski CF, Covey DF, Mennerick S. Neurosteroid access to the $GABA_A$ receptor. J Neurosc. 2005; 25:11605–11613.
73. Pinault D. The thalamic reticular nucleus: structure, function and concept. Brain Res Rev. 2004; 46:1–31. [PubMed: 15297152] 73 Uzunova V, Sampson L, Uzunov DP. Relevance of endogenous 3a-reduced neurosteroids to depression and antidepressant action. Psychopharmacology. 2006; 186:351–361. [PubMed: 16249906]
74. Matsumoto K, Uzunova V, Pinna G, Taki K, Uzunov DP, Watanabe H, Mienvielle J-M, Guidotti A, Costa E. Permissive role of brain allopregnanolone content in the regulation of pentobarbital-induced righting reflex loss. Neuropharmacology. 1999; 38:955–963. [PubMed: 10428414]
75. Serra M, Pisu MG, Littera M, Papi G, Sanna E, Tuveri F, Usala L, Purdy RH, Biggio G. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J Neurochem. 2000; 75:732–40. [PubMed: 10899949]
76. Bortolato M, Devoto P, Roncada P, Frau R, Flore G, Saba P, Pistritto G, Soggiu A, Pisanu S, Zappala A, Ristaldi MS, Tattoli M, Cuomo V, Marrosu F, Barbaccia ML. Isolation rearing induced reduction of brain 5α-reductase expression: Relevance to dopaminergic impairments. Neuropharmacology. 2011 Jan 20. [Epub ahead of print].
77. Matsumoto K, Puia G, Dong E, Pinna G. $GABA_A$ receptor neurotransmission dysfunction in a mouse model of social isolation-induced stress: possible insights into a nonserotonergic mechanism of action of SSRIs in mood and anxiety disorders. Stress. 2007; 10:3–12. [PubMed: 17454962]
78. Dong E, Matsumoto K, Uzunova V, Sugaya I, Costa E, Guidotti A. Brain 5α-dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. Proc Natl Acad Sci USA. 2001; 98:2849–2854. [PubMed: 11226329]
79. Pibiri F, Nelson M, Guidotti A, Costa E, Pinna G. Decreased allopregnanolone content during social isolation enhances contextual fear: a model relevant for posttraumatic stress disorder. Proc Natl Acad Sci USA. 2008; 105:5567–5572. [PubMed: 18391192]
80. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, Gavish M. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. Trends Pharmacol Sci. 2006; 27:402–9. [PubMed: 16822554]

81. Nelson M, Pinna G. S-norfluoxetine infused into the basolateral amygdala increases allopregnanolone levels and reduces aggression in socially isolated mice. Neuropharmacology. 2011; 60:1154–1159. [PubMed: 20971127]
82. Rodgers RJ, Johnson NJ. Behaviorally selective effects of neuroactive steroids on plus-maze anxiety in mice. Pharmacol Biochem Behav. 1998; 59:221–32. [PubMed: 9443559]
83. Frye CA, Rhodes ME. Infusions of 5alpha-pregnan-3alpha-ol-20-one (3alpha,5alpha- THP) to the ventral tegmental area, but not the substantia nigra, enhance exploratory, antianxiety, social and sexual behaviours and concomitantly increase 3alpha,5alpha-THP concentrations in the hippocampus, diencephalon and cortex of ovariectomised oestrogenprimed rats. J Neuroendocrinol. 2006; 18:960–75. [PubMed: 17076771]
84. Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res. 1991; 561:157–61. [PubMed: 1686744]
85. Wieland S, Lan NC, Mirasedeghi S, Gee KW. Anxiolytic activity of the progesterone metabolite 5 alpha-pregnan-3 alpha-o1–20-one. Brain Res. 1991; 565:263–8. [PubMed: 1688192]
86. Deo GS, Dandekar MP, Upadhya MA, Kokare DM, Subhedar NK. Neuropeptide Y Y1 receptors in the central nucleus of amygdala mediate the anxiolytic-like effect of allopregnanolone in mice: Behavioral and immunocytochemical evidences. Brain Res. 2010; 1318:77–86. [PubMed: 20059983]
87. Engin E, Treit D. The anxiolytic-like effects of allopregnanolone vary as a function of intracerebral microinfusion site: the amygdala, medial prefrontal cortex, or hippocampus. Behav Pharmacol. 2007; 18:461–70. [PubMed: 17762514]
88. Frye CA, Paris JJ, Rhodes ME. Increasing 3alpha,5alpha-THP following inhibition of neurosteroid biosynthesis in the ventral tegmental area reinstates anti-anxiety, social, and sexual behavior of naturally receptive rats. Reproduction. 2009; 137:119–28. [PubMed: 18818272]
89. Akbarian S, Huntsman MM, Kim JJ, Tafazzoli A, Potkin SG, Bunney WE Jr, Jones EG. $GABA_A$ receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. Cereb Cortex. 1995; 5:550–60. [PubMed: 8590827]
90. Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, Opeskin K, Copolov DL. Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J Neurochem. 1999; 72:1593–9. [PubMed: 10098866]
91. Lewis DA. GABAergic local circuit neurons and prefrontal cortical dysfunction in schizophrenia. Brain Res Brain Res Rev. 2000; 31:270–6. [PubMed: 10719153]
92. Ishikawa M, Mizukami K, Iwakiri M, Hidaka S, Asada T. $GABA_A$ receptor gamma subunits in the prefrontal cortex of patients with schizophrenia and bipolar disorder. Neuroreport. 2004; 15:1809–12. [PubMed: 15257153]
93. Rudolph U, Möhler H. Analysis of GABAA receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol. 2004; 44:475–98. [PubMed: 14744255]
94. Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Möhler H. Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. Nature. 1999; 401:796–800. [PubMed: 10548105]
95. Pinna G, Agis-Balboa RC, Zhubi A, Matsumoto K, Grayson DR, Costa E, Guidotti A. Imidazenil and diazepam increase locomotor activity in mice exposed to protracted social isolation. Proc Natl Acad Sci USA. 2006; 103:4275–4280. [PubMed: 16537521]
96. Crestani F, Martin JR, Möhler H, Rudolph U. Mechanism of action of the hypnotic zolpidem in vivo. Br J Pharmacol. 2000; 131:1251–4. [PubMed: 11090095]
97. Smith SS, Gong QH, Hsu FC, Markowitz RS, ffrench-Mullen JM, Li X. GABA(A) receptor alpha4 subunit suppression prevents withdrawal properties of an endogenous steroid. Nature. 1998; 392:926–30. [PubMed: 9582073]
98. Valzelli L. The “isolation syndrome” in mice. Psychopharmacologia. 1973; 31:305–20. [PubMed: 4582344] 99 Valzelli L. Psychopharmacology of aggression: an overview. Int Pharmacopsychiatry. 1981; 16:39–48. [PubMed: 7298270]

99. Matsumoto K, Cai B, Satoh T, Ohta H, Watanabe H. Desipramine enhances isolationinduced aggressive behavior in mice. Pharmacol Biochem Behav. 1991; 39:167–70. [PubMed: 1924499]
100. Fish EW, De Bold JF, Miczek KA. Aggressive behavior as a reinforcer in mice: activation by allopregnanolone. Psychopharmacology (Berl). 2002; 163:459–66. [PubMed: 12373446]
101. Engel SR, Grant KA. Neurosteroids and behavior. International Review of Neurobiology. 2001; 46:321–348. [PubMed: 11599304]
102. Dranovsky A, Picchini AM, Moadel T, Sisti AC, Yamada A, Kimura S, Leonardo ED, Hen R. Experience dictates stem cell fate in the adult hippocampus. Neuron. 2011 Jun 9; 70(5):908–23. [PubMed: 21658584]
103. Kempermann G, Kronenberg G. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol Psychiatry. 2003; 54:499–503. [PubMed: 12946878]
104. Coyle JT, Duman RS. Finding the intracellular signaling pathways affected by mood disorder treatments. Neuron. 2003; 38:157–60. [PubMed: 12718851]
105. Dranovsky A, Hen R. Hippocampal neurogenesis: regulation by stress and antidepressants. Biol Psychiatry. 2006; 59:1136–43. [PubMed: 16797263]
106. Kempermann G, Gage FH. Closer to neurogenesis in adult humans. Nat Med. 1998; 4:555–7. [PubMed: 9585224]
107. van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999; 96:13427–31. [PubMed: 10557337]
108. Leuner B, Mendolia-Loffredo S, Kozorovitskiy Y, Samburg D, Gould E, Shors TJ. Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. J Neurosci. 2004; 24:7477–81. [PubMed: 15329394]
109. David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, Drew M, Craig DA, Guiard BP, Guilloux JP, Artymyshyn RP, Gardier AM, Gerald C, Antonijevic IA, Leonardo ED, Hen R. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron. 2009; 62:479–93. [PubMed: 19477151]
110. Wang JM, Liu L, Irwin RW, Chen S, Brinton RD. Regenerative potential of allopregnanolone. Brain Res Rev. 2008; 57:398–409. [PubMed: 17931704]
111. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, Weisstaub N, Lee J, Duman R, Arancio O, Belzung C, Hen R. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003; 301:805–9. [PubMed: 12907793]
112. Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, Arango V. Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology. 2009; 34:2376–89. [PubMed: 19606083]
113. Castro JE, Varea E, Márquez C, Cordero MI, Poirier G, Sandi C. Role of the amygdala in antidepressant effects on hippocampal cell proliferation and survival and on depression-like behavior in the rat. PLoS One. 2010; 5:e8618. [PubMed: 20062812]
114. Wong EY, Herbert J. The corticoid environment: a determining factor for neural progenitors' survival in the adult hippocampus. Eur J Neurosci. 2004; 20:2491–8. [PubMed: 15548194]
115. Karishma KK, Herbert J. Dehydroepiandrosterone (DHEA) stimulates neurogenesis in the hippocampus of the rat, promotes survival of newly formed neurons and prevents corticosterone-induced suppression. Eur J Neurosci. 2002; 16:445–53. [PubMed: 12193187]
116. Pinnock SB, Lazic SE, Wong HT, Wong IH, Herbert J. Synergistic effects of dehydroepiandrosterone and fluoxetine on proliferation of progenitor cells in the dentate gyrus of the adult male rat. Neuroscience. 2009; 158:1644–51. [PubMed: 19068226]
117. Zhang Z, Yang R, Zhou R, Li L, Sokabe M, Chen L. Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. Hippocampus. 2010; 20:402–12. [PubMed: 19475650]
118. Wang JM, Johnston PB, Ball BG, Brinton RD. The neurosteroid allopregnanolone promotes proliferation of rodent and human neural progenitor cells and regulates cell-cycle gene and protein expression. J Neurosci. 2005; 25:4706–18. [PubMed: 15888646]

119. Wang JM, Singh C, Liu L, Irwin RW, Chen S, Chung EJ, Thompson RF, Brinton RD. Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. Proc Natl Acad Sci U S A. 2010; 107:6498–503. [PubMed: 20231471]
120. Brinton RD, Wang JM. Preclinical analyses of the therapeutic potential of allopregnanolone to promote neurogenesis in vitro and in vivo in transgenic mouse model of Alzheimer's disease. Curr Alzheimer Res. 2006; 3:11–7. [PubMed: 16472197]
121. Keller EA, Zamparini A, Borodinsky LN, Gravielle MC, Fiszman ML. Role of allopregnanolone on cerebellar granule cells neurogenesis. Brain Res Dev Brain Res. 2004; 153:13–7.
122. Papadopoulos V, Lecanu L. CAPROSPINOL: Discovery of a steroid drug candidate to treat Alzheimer's disease based on 22R-hydroxycholesterol structure and properties. J Neuroendocrinol. 201110.1111/j.1365–2826.2011.02167.x
123. Nagahara AH, Tuszynski MH. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. Nat Rev Drug Discov. 2011; 10:209–19. [PubMed: 21358740]
124. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003; 112:257–69. [PubMed: 12553913]
125. Quirk GJ, Mueller D. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology. 2008; 33:56–72. [PubMed: 17882236]
126. Quirk GJ, Milad MR. Neuroscience: Editing out fear. Nature. 2010; 463:36–7. [PubMed: 20054384]
127. Bredy TW, Wu H, Crego C, Zellhoefer J, Sun YE, Barad M. Histone modifications around individual BDNF gene promoters in prefrontal cortex are associated with extinction of conditioned fear. Learn Mem. 2007; 14:268–76. [PubMed: 17522015]
128. Sen S, Duman R, Sanacora G. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. Biol Psychiatry. 2008; 64:527–32. [PubMed: 18571629]
129. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A. 1996; 93:3908–13. [PubMed: 8632988]
130. Sheline YI, Mittler BL, Mintun MA. The hippocampus and depression. Eur Psychiatry. 2002; (Suppl 3):300–5. [PubMed: 15177085]
131. Schmahl C, Berne K, Krause A, Kleindienst N, Valerius G, Vermetten E, Bohus M. Hippocampus and amygdala volumes in patients with borderline personality disorder with or without posttraumatic stress disorder. J Psychiatry Neurosci. 2009; 34:289–95. [PubMed: 19568480]
132. Acheson DT, Gresack JE, Risbrough VB. Hippocampal dysfunction effects on context memory: Possible etiology for posttraumatic stress disorder. Neuropharmacology. 2011 May 11.
133. Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry. 2003; 54:693–702. [PubMed: 14512209]
134. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry. 2003; 54:70–5. [PubMed: 12842310]
135. Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry. 2001; 50:260–5. [PubMed: 11522260]
136. Nelson M, Pibiri F, Pinna G. Relationship between allopregnanolone (Allo) and reduced brain BDNF and reelin expression in a mouse model of posttraumatic stress disorders (PTSD). Society for Neuroscience. 2010:Abstract, 666.22.
137. Tueting, P.; Pinna, G. Behavior associated with an enriched environment and with social isolation in mice. Society for Neuroscience Abstract. Poster; Orlando, FL. November 2–7, 2002; p. 106.12
138. Martinez, LA.; Pibiri, F.; Nelson, M.; Nin, MS.; Pinna, G. Selective brain steroidogenic stimulant (SBSS)-induced neurosteroid upregulation facilitates fear extinction and reinstatement of fear

memory by a BDNF-mediated mechanism in a mouse model of PTSD. Society for Neuroscience Abstract; Washington, DC. November 12–16, 2011;

139. Sanacora G, Saricicek A. GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action. CNS Neurol Disord Drug Targets. 2007; 6:127–140. [PubMed: 17430150]
140. Sanacora G. Cortical Inhibition, gamma-aminobutyric acid, and major depression: there is plenty of smoke but is there fire? Biological Psychiatry. 2010; 67:397–8. [PubMed: 20159143]
141. Bremner JD, Innis RB, Southwick SM, Staib L, Zoghbi S, Charney DS. Decreased benzodiazepine receptor binding in prefrontal cortex in combat-related posttraumatic stress disorder. Am J Psychiatry. 2000; 157:1120–6. [PubMed: 10873921] 104 Fujita M, Southwick SM, Denucci CC, Zoghbi SS, Dillon MS, Baldwin RM, Bozkurt A.
142. Kugaya A, Verhoeff NP, Seibyl JP, Innis RB. Central type benzodiazepine receptors in Gulf War veterans with posttraumatic stress disorder. Biol Psychiatry. 2004; 56:95–100. [PubMed: 15231441]
143. Vaiva G, Thomas P, Ducrocq F, Fontaine M, Boss V, Devos P, Rascle C, Cottencin O, Brunet A, Laffargue P, Goudemand M. Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder. Biol Psychiatry. 2004; 55:250–4. [PubMed: 14744465]
144. Feusner J, Ritchie T, Lawford B, Young RM, Kann B, Noble EP. GABA(A) receptor beta 3 subunit gene and psychiatric morbidity in a post-traumatic stress disorder population. Psychiatry Res. 2001; 104:109–17. [PubMed: 11711165]
145. Spivak B, Maayan R, Kotler M, Mester R, Gil-Ad I, Shtaif B, Weizman A. Elevated circulatory level of $GABA_A$-antagonistic neurosteroids in patients with combat-related posttraumatic stress disorder. Psychol Med. 2000; 30:1227–1231. [PubMed: 12027057]
146. Sondergaard HP, Hansson L-O, Theorell T. Elevated blood levels of dehydroepiandrosterone sulphate vary with symptom load in posttraumatic stress disorder: findings from a longitudinal study of refugees in Sweden. Psychother Psychosom. 2002; 71:298–303. [PubMed: 12207110]
147. Rasmusson AM, Vasek J, Lipschitz DS, Vojvoda D, Mustone ME, Shi Q, Gudmundsen G, Morgan CA, Wolfe J, Charney DS. An increased capacity for adrenal DHEA release is associated with decreased avoidance and negative mood symptoms in women with PTSD. Neuropsychopharm. 2004; 29:1546–57.
148. Brady K, Pearlstein T, Asnis GM, Baker D, Rothbaum B, Sikes C, Farfel GM. Efficacy and safety of sertraline treatment of posttraumatic stress disorder. JAMA. 2000; 283:1837–1844. [PubMed: 10770145]
149. Davidson JRT, Rothbaum BO, van der Kolk BA, Sikes CR, Farfel GM. Multicenter, double-blind comparison of sertraline and placebo in the treatment of posttraumatic stress disorder. Arch Gen Psychiatry. 2001; 58:485–492. [PubMed: 11343529]
150. Marshall RD, Beebe KL, Oldham M, Zaninelli R. Efficacy and safety of paroxetinetreatment for PTSD: A fixed-dose, placebo-controlled study. Am J Psychiatry. 2001; 158:1982–1988. [PubMed: 11729013]
151. Tucker P, Zaninelli R, Yehuda R, Ruggiero L, Dillingham K, Pitts CD. Paroxetine in the treatment of chronic posttraumatic stress disorder: Results of a placebo-controlled, flexibledosage study. J Clin Psychiatry. 2001; 62:860–868. [PubMed: 11775045]
152. Friedman MJ, Marmar CR, Baker DG, Sikes CR, Farfel GM. Randomized, doubleblind comparison of sertraline and placebo for posttraumatic stress disorder in a Department of Veterans Affairs setting. J Clin Psychiatry. 2007; 68:711–720. [PubMed: 17503980]
153. Mtchedlishvili Z, Kapur J. High-affinity, slowly desensitizing $GABA_A$ receptors mediate tonic inhibition in hippocampal dentate granule cells. Molecular Pharm. 2006; 69:564–75.
154. Ressler KJ, Paschall G, Zhou XL, Davis M. Regulation of synaptic plasticity genes during consolidation of fear conditioning. J Neurosci. 2002; 22:7892–902. [PubMed: 12223542]
155. Myers KM, Davis M. Mechanisms of fear extinction. Molecular Psychiatry. 2007; 12:120–50. [PubMed: 17160066]
156. Barbaccia ML, Serra M, Purdy RH, Biggio G. Stress and neuroactive steroids. Int Rev Neurobiol. 2001; 46:243–72. [PubMed: 11599302]

157. Breslau N, Chase GA, Anthony JC. The uniqueness of the DSM definition of post-traumatic stress disorder: implications for research. Psychol Med. 2002; 32(4):573–6. [PubMed: 12102371]
158. Zhao H, Mayhan WG, Arrick DM, Xiong W, Sun H. Alcohol-induced exacerbation of ischemic brain injury: role of NAD(P)H oxidase. Alcoholism: Clinical & Experimental Research. 2010; 34:1948–55.
159. Cagetti E, Pinna G, Guidotti A, Baicy K, Olsen RW. Chronic intermittent ethanol (CIE) administration in rats decreases levels of neurosteroids in hippocampus, accompanied by altered behavioral responses to neurosteroids and memory function. Neuropharmacology. 2004; 46:570–9. [PubMed: 14975681]
160. Penning TM, Jin Y, Steckelbroeck S, Lanisnik Rizner T, Lewis M. Structure–function of human 3 -hydroxysteroid dehydrogenases genes and proteins. Mol Cell Endocrin. 2004; 215:63–72.
161. Penning TM. 3α-Hydroxysteroid dehydrogenase: three dimensional structure and gene regulation. J Endocrinology. 1996; 150:5175–5187.
162. Tomkins GM. Enzymatic mechanisms of hormone metabolism. I. Oxidation-reduction of the steroid nucleus. Recent Prog Horm Res. 1956; 12:125–33. [PubMed: 13401053]
163. Hou YT, Lin HK, Penning TM. Dexamethasone regulation of the rat 3 - hydroxysteroid/ dihydrodiol dihydrogenase (3α-HSD/DD) gene. Mol Pharmacol. 1998; 53:459–466. [PubMed: 9495812]
164. Griffin LD, Mellon SH. Selective serotonin reuptake inhibitors directly alter activity of neurosteroidogenic enzymes. Proc Natl Acad Sci USA. 1999; 96:13512–7. [PubMed: 10557352]
165. Trauger JW, Jiang A, Stearns BA, LoGrasso PV. Kinetics of allopregnanolone formation catalyzed by human 3 alpha hydroxysteroid dehydrogenase TypeIII (AKR1C2). Biochemistry. 2002; 41:13451–13459. [PubMed: 12416991]
166. Chung ES, Chung YC, Boka E, Baik HH, Park ES, Parke J-Y, Yoon SH, Jin BK. Fluoxetine prevents LPS-induced degeneration of nigral dopaminergic neurons by inhibiting microglia-mediated oxidative stress. Brain Res. 2010:143–150. [PubMed: 20858471]
167. Gulinello M, Gong QH, Smith SS. Progesterone withdrawal increases the anxiolytic actions of gaboxadol: role of alpha4betadelta GABA(A) receptors. Neuroreport. 2003; 14:43–46. [PubMed: 12544828]
168. Kaminski RM, Livingood MR, Rogawski MA. Allopregnanolone analogs that positively modulate GABA receptors protect against partial seizures induced by 6-Hz electrical stimulation in mice. Epilepsia. 2004; 45:864–7. [PubMed: 15230714]
169. Berlant JL. Prospective open-label study of add-on and monotherapy topiramate in civilians with chronic nonhallucinatory posttraumatic stress disorder. BMC Psychiatry. 2004; 4:24. [PubMed: 15315714]
170. Johnson BA, Ait-Daoud N, Akhtar FZ, Javors MA. Use of oral topiramate to promote smoking abstinence among alcohol-dependent smokers: a randomized controlled trial. Arch Int Med. 2005; 165:1600–5. [PubMed: 16043677]
171. Marx CE, VanDoren MJ, Duncan GE, Lieberman JA, Morrow AL. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. Neuropsychopharm. 2003; 28:1–13.
172. Genazzani AD, Stomati M, Bernardi F, Pieri M, Rovati L, Genazzani AR. Longterm low-dose dehydroepiandrosterone oral supplementation in early and late postmenopausal women modulates endocrine parameters and synthesis of neuroactive steroids. Fertility & Sterility. 2003; 80:1495–501. [PubMed: 14667889]
173. Schmidt PJ, Daly RC, Bloch M, Smith MJ, Danaceau MA, Simpson St Clair L, Murphy JH, Haq N, Rubinow DR. Dehydroepiandrosterone monotherapy in midlife-onset major and minor depression. Arch Gen Psychiatry. 2005; 62:154–162. [PubMed: 15699292]
174. Hamner MB. Clozapine treatment for a veteran with psychosis and PTSD. Am J Psychiaty. 1996; 153:841.
175. Petty F, Brannan S, Casada J, Davis LL, Gajewski V, Kramer GL, Stone RC, Teten AL, Worchel J, Young KA. Olanzapine treatment for post-traumatic stress disorder: an open-label study. Int Clin Psychopharmacology. 2001; 16:331–337.

176. Barbaccia ML. Neurosteroidogenesis: relevance to neurosteroid actions in brain and modulation by psychotropic drugs. Crit Rev Neurobiol. 2004; 16:67–74. [PubMed: 15581401]

177. Suris A, North C, Adinoff B, Powell CM, Greene R. Effects of exogenous glucocorticoid on combat-related PTSD symptoms Ann clin. Psychiatry. 2010; 22:274–9.

178. Brandes RP. A Radical Adventure: The Quest for Specific Functions and Inhibitors of Vascular NAPDH Oxidases, Circ. Res. 2003; 92:583–585.

179. Rasmusson AM, Schnurr P, Zukowska Z, Scioli E, Forman DE. Adaptation to Extreme Stress: PTSD, NPY, and Metabolic Syndrome. Experimental Biology and Medicine. 2010; 235:1150–6. [PubMed: 20881319]

180. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. Front Neuroendocrinol. 2000; 21:1–56. [PubMed: 10662535]

181. Reddy DS, Kulkarni SK. Role of GABA-A and mitochondrial diazepam binding inhibitor receptors in the anti-stress activity of neurosteroids in mice. Psychopharmacology. 1996; 128:280–92. [PubMed: 8972548]

182. Romeo E, Auta J, Kozikowski AP, Ma D, Papadopoulos V, Puia G, Costa E, Guidotti A. 2-Aryl-3-indoleacetamides (FGIN-1): a new class of potent and specific ligands for the mitochondrial DBI receptor (MDR). J Pharmacol Exp Ther. 1992; 262:971–8. [PubMed: 1326631]

183. Korneyev A, Pan BS, Polo A, Romeo E, Guidotti A, Costa E. Stimulation of brain pregnenolone synthesis by mitochondrial diazepam binding inhibitor receptor ligands in vivo. J Neurochem. 1993; 61:1515–24. [PubMed: 8397297]

184. Kita A, Kohayakawa H, Kinoshita T, Ochi Y, Nakamichi K, Kurumiya S, Furukawa K, Oka M. Antianxiety and antidepressant-like effects of AC-5216, a novel mitochondrial benzodiazepine receptor ligand. Br J Pharmacol. 2004; 142:1059–72. [PubMed: 15249420]

185. Girard C, Liu S, Cadepond F, Adams D, Lacroix C, Verleye M, Gillardin JM, Baulieu EE, Schumacher M, Schweizer-Groyer G. Etifoxine improves peripheral nerve regeneration and functional recovery. Proc Natl Acad Sci U S A. 2008; 105(51):20505–10. [PubMed: 19075249]

186. Peters J, Dieppa-Perea LM, Melendez LM, Quirk GJ. Induction of fear extinction with hippocampal-infralimbic BDNF. Science. 2010; 328:1288–90. [PubMed: 20522777]

187. Maguire J, Mody I. Steroid hormone fluctuations and $_{GABAA}$ Receptor plasticity. Psychoneuroendocrinology. 2009; 34(Suppl 1):S84–90. [PubMed: 19632051]