



## The role of allopregnanolone in depressive-like behaviors: Focus on neurotrophic proteins

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### ABSTRACT

Allopregnanolone (3 $\alpha$ ,5 $\alpha$ -tetrahydroprogesterone; pharmaceutical formulation: brexanolone) is a neurosteroid that has recently been approved for the treatment of postpartum depression, promising to fill part of a long-lasting gap in the effectiveness of pharmacotherapies for depressive disorders. In this review, we explore the experimental research that characterized the antidepressant-like effects of allopregnanolone, with a particular focus on the neurotrophic adaptations induced by this neurosteroid in preclinical studies. We demonstrate that there is a consistent decrease in allopregnanolone levels in limbic brain areas in rodents submitted to stress-induced models of depression, such as social isolation and chronic unpredictable stress. Further, both the drug-induced upregulation of allopregnanolone or its direct administration reduce depressive-like behaviors in models such as the forced swim test. The main drugs of interest that upregulate allopregnanolone levels are selective serotonin reuptake inhibitors (SSRIs), which present the neurosteroidogenic property even in lower, non-SSRI doses. Finally, we explore how these antidepressant-like behaviors are related to neurogenesis, particularly in the hippocampus. The protagonist in this mechanism is likely the brain-derived neurotrophic factor (BDNF), which is decreased in animal models of depression and may be restored by the normalization of allopregnanolone levels. The role of an interaction between GABA and the neurotrophic mechanisms needs to be further investigated.

### 1. Introduction

Depression (also referred to as ‘major depression’ or ‘major depressive disorder’) is a highly prevalent mental illness that is estimated to affect up to nearly four percent of the world population and is the leading cause of disability worldwide (Rehm and Shield, 2019). It is clinically characterized mainly by its core symptoms of depressed mood and anhedonia, but a wide array of accompanying secondary symptoms render depression a rather heterogeneous disorder regarding its phenotype (Bentley et al., 2014). This heterogeneity is also reflected in its etiology, which is likely responsible for the disappointingly low success rate (around 50%) of widely prescribed antidepressants that act by increasing brain monoamine levels when these drugs are confronted against placebo in clinical trials (Vries et al., 2018). Thus, it becomes

evident that additional neurotransmitter systems are deeply involved with depression, and that molecular targets other than monoamine modulators must be pursued in order to achieve a more complete efficacy in the pharmacological treatment for depression.

Evidence of the relationship of the GABAergic system with mood disorders dates as far back as 1980 when treatment with valproic acid was reported to show positive effects for bipolar disorder (Emrich et al., 1980). In fact, GABA levels are diminished in the brain of depressed patients (Sanacora et al., 2004), and the stimulation of GABAergic transmission has been proposed as a novel strategy for the treatment of depression, particularly through stimulation of the GABA type A receptor (GABA<sub>A</sub>R) (Lüscher and Möhler, 2019). The GABA<sub>A</sub>R is one of the main receptors for GABA and consists of an ion-gated channel that hyperpolarizes the postsynaptic neuron when activated (Chua and

**Abbreviations:** BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; CUS, chronic unpredictable stress; EKR, extracellular signal-regulated kinase; FST, forced swim test; GABA,  $\gamma$ -aminobutyric acid; GABA<sub>A</sub>R, GABA type A receptor; HSD, hydroxysteroid dehydrogenase; NGF, nerve growth factor; PTSD, post-traumatic stress disorder; PXR, pregnane xenobiotic receptor; SBSS, selective brain steroidogenic stimulant; SSRI, selective serotonin reuptake inhibitor; THP, tetrahydroprogesterone; TrkB, tropomyosin receptor kinase B; TSPO, 18 kDa translocator protein; USV, ultrasonic vocalization

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**Table 1**  
Brain allopregnanolone levels in animal models of depression.

Published in	Spe-cies	Sex (es)	Model(s)		Behavioral test (s)	Brain areas(s)	Main findings	
			Name(s)	Dura-tion			Behavioral changes	Brain allopregnanolone levels
<b>Social isolation</b>								
Matsumoto et al. (1999)	Mice	♂	SI	2–10 wks	None	● FC	N/A	● SI (6–10 wks): ↓
Serra et al. (2000)	Rats	♂	SI SI + HDL SI→FSS	1–30 d 30 d 30 d	None	● CTX	N/A	● SI (1 or 2 d): ≅ ● SI (7 or 30 d): ↓ ● SI + HDL: ≅ ● FSS: ↑ (↑ <sub>SI</sub> > ↑ <sub>GH</sub> ) ● SI (4–8 wks): ↓ ● ↓
Pinna et al. (2003)	Mice	♂	SI	1 d–8 wks	RIT	● OB	● SI (4–8 wks): ↑ <sub>aggression</sub>	● SI: ↓ in ♂ <sub>sham</sub>
Pinna et al. (2004b)	Mice	♂	SI	4–6 wks	None	● OB ● FC ● OB	N/A	● ORX: ≅ ● SI + ORX: ↓ ● SI: ≅ in ♀ <sub>sham</sub> ● SI + TP: ↓ in ♀ <sub>sham</sub> ● SI + OVX: ≅ ● SI + OVX + TP: ↓ ● SI: ≅ ● SI + TP: ↓
Pinna et al. (2005)	Mice	♂ + ♀	SI	3 wks	RIT	● OB	● SI: ↑ <sub>aggression</sub> in ♂ <sub>sham</sub> ● ORX: ≅ <sub>aggression</sub> ● ORX + TP: ↑ <sub>aggression</sub> ● SI: ≅ <sub>aggression</sub> in ♀ <sub>sham</sub> ● SI + TP: ↑ <sub>aggression</sub> in ♀ <sub>sham</sub> ● SI + OVX: ≅ <sub>aggression</sub> ● SI + OVX + TP: ↑ <sub>aggression</sub> ● SI ↑ <sub>aggression</sub> in ♂ ● SI ≅ <sub>aggression</sub> in ♀ ● SI + TP: ↑ <sub>aggression</sub> in ♀ ● Aggression (SI vs. GH): N.I.	● SI: ↓ in ♂ <sub>sham</sub> ● ORX: ≅ ● SI + ORX: ↓ ● SI: ≅ in ♀ <sub>sham</sub> ● SI + TP: ↓ in ♀ <sub>sham</sub> ● SI + OVX: ≅ ● SI + OVX + TP: ↓ ● SI: ≅ ● SI + TP: ↓
Pibiri et al. (2006)	Mice	♂ + ♀	SI	3 wks	RIT	● OB ● FC	● SI ↑ <sub>aggression</sub> in ♂ ● SI ≅ <sub>aggression</sub> in ♀ ● SI + TP: ↑ <sub>aggression</sub> in ♀	● SI: ↓ in OB, FC, BLA and HPC ● SI: ≅ in STR
Nelson and Pinna (2011)	Mice	♂	SI	4 wks	RIT	● OB ● FC ● BLA ● HPC ● STR ● HPC	● SI (6 and 10 wks): ↑ <sub>immobility</sub> ● SI: ↑ <sub>novel food remaining</sub> ● No changes in the OFT	● SI: ↓ in OB, FC, BLA and HPC ● SI: ≅ in STR
Evans et al. (2012)	Rats	♂	SI <sub>maint.</sub> SI <sub>rescue</sub>	6 wks 10 wks	FST NSFT OFT	● HPC	● SI (6 and 10 wks): ↑ <sub>immobility</sub> ● SI: ↑ <sub>novel food remaining</sub> ● No changes in the OFT	● SI (6 and 10 wks): ↓
Pibiri et al. (2008)	Mice	♂	SI	3–4 wks	CFCT	● OB ● FC ● AMY ● STR ● HPC ● CBL ● CTX	● SI: ↑ <sub>freezing</sub>	● ↓ in OB, FC, AMY and HPC ● ≅ in STR and CBL
Pisu et al. (2016)	Rats	♂ + ♀	SI SI→FSS	30 d 30 d	SPT	● CTX	● SI ↓ <sub>preference</sub> in ♂ ● SI ≅ <sub>preference</sub> in ♀	● SI: (↓ <sub>♂</sub> > ↓ <sub>♀</sub> ) ● FSS: ↑ in ♂ (↑ <sub>GH</sub> < ↑ <sub>SI</sub> ) ● FSS: ≅ in ♀ (GH = SI)
<b>Other animal models</b>								
Uzunova et al. (2003)	Rats	♂	OBX	N/A	None	● WB ● FC ● HPC ● CTX ● AMY	N/A	● ↑ WB at 7 and 28 d ● ↓ in AMY and FC at 7 and 14 d ● ↑ in CTX at 7 and 14 d ● ↓ <sub>tend.</sub> in HPC at 14 d ● ↓
Uzunova et al. (2004)	Rats	♂	OBX	5 wks	None	● CTX	N/A	● L-UVL > H-UVL in ♂ ● L-UVL > H-UVL in ♀ <sub>diestrus</sub> ● L-UVL = H-UVL in ♀ <sub>proestrus</sub>
Zimmerberg et al. (2005)	Rats	♂ + ♀	H-UVL L-UVL	N/A	FST SIT DBT OFT	● HPC + AMY	● Immobility: L-UVL < H-UVL ● SIT: L-UVL > H-UVL in ♀ <sub>proestrus</sub> ● Center area: L-UVL > H-UVL ● No changes in the OFT	● L-UVL > H-UVL in ♂ ● L-UVL > H-UVL in ♀ <sub>diestrus</sub> ● L-UVL = H-UVL in ♀ <sub>proestrus</sub>
Zhang et al., 2014b	Rats	♂	TDS	Single event	CFCT EPM SCT	● PFC	● ↑ <sub>freezing</sub> ● ↓ <sub>time in open arms</sub> ● ↑ <sub>rearrings</sub>	● ↓
Qiu et al. (2016)	Rats	♂	DM1	2 wks	FST SPT NSFT OFT	● HPC ● PFC	● ↑ <sub>immobility</sub> ● ↓ <sub>preference</sub> ● ↑ <sub>latency to feed</sub> ● No changes in the OFT	● ↓
Zhang et al. (2017)	Rats	♂	CUS	4 wks	SPT NSFT OFT	● HPC ● PFC	● ↓ <sub>preference</sub> ● ↑ <sub>latency to feed</sub> ● ↓ <sub>rearrings</sub> ● ↓ <sub>crossings</sub>	● ↓
Qiu et al. (2017)	Rats	♂	CUS	4 wks	FST SPT NSFT OFT	● HPC ● PFC	● ↑ <sub>immobility</sub> ● ↓ <sub>preference</sub> ● ↑ <sub>latency to feed</sub> ● No changes in the OFT	● ↓
Guo et al. (2017)	Rats	♂	CUS	3 wks	SPT NSFT OFT	● HPC ● AMY	● ↓ <sub>preference</sub> ● ↑ <sub>latency to feed</sub> ● ↓ <sub>perm. center</sub> ● ↓ <sub>distance</sub>	● ↓ in HPC ● ↓ in AMY
Lee et al. (2018)	Rats	♂	SPS	Single event	CFCT OFT	● HPC ● PFC	● ↑ <sub>freezing</sub> ● No changes in the OFT	● ↓

(continued on next page)

Table 1 (continued)

Published in	Spe-cies	Sex (es)	Model(s)		Behavioral test (s)	Brain areas(s)	Main findings	
			Name(s)	Dura-tion			Behavioral changes	Brain allopregnanolone levels
Xu et al. (2018a)	Rats	♂	CUS	4 wks	EPM NSFT OFT	● HPC ● PFC	● ↓time in open arms ● ↑latency to feed ● No changes in the OFT	• ↓
Xu et al. (2018b)	Rats	♂	SPS	Single event	CFCT EPM OFT	● HPC ● PFC ● AMY	● ↑freezing ● ↓time in open arms ● No changes in the OFT	• ↓
Zhang et al. (2018)	Mice	♂	TDS	Single event	CFCT EPM OFT	● DG	● ↑freezing ● ↓time in open arms ● No changes in the OFT	• ↓
Su et al. (2019)	Rats	♂	SPS	Single event	CFCT EPM OFT	● HPC ● PFC	● ↑freezing ● ↓time in open arms ● No changes in the OFT	• ↓

Abbreviations and legends: increases (↑); decreases (↓); does not change (≅); followed by (→); tendency (tend.); whole brain (WB); cerebral cortex (CTX); hippocampus (HPC); dentate gyrus (DG); prefrontal cortex (PFC); frontal cortex (FC); amygdala (AMY); basolateral amygdala (BLA); striatum (STR); cerebellum (CBL); olfactory bulb (OB); olfactory bulbectomy (OBX); ovariectomy (OVX); orchietomy (ORX); testosterone propionate (TP); diabetes mellitus type 1 (DM1); chronic unpredictable stress (CUS); high ultrasonic vocalization line (H-UVL); low ultrasonic vocalization line (L-UVL); social isolation (SI); group housed (GH); foot-shock stress (FSS); single prolonged stress (SPS); time-dependent sensitization (TDS); handling (HDL); maintenance (maint.); contextual fear conditioning test (CFCT); elevated plus maze (EPM); sucrose preference test (SPT); novelty-suppressed feeding test (NSFT); forced swim test (FST); open field test (OFT); resident-intruder test (RIT); defensive burying test (DBT); social interaction test (SIT); staircase test (SCT); not applicable (N/A); not informed (N.I.); \*except if noted otherwise. Unless otherwise specified, changes in allopregnanolone levels refer to all interventions used (model or drugs), doses, or brain areas analyzed in each study.

Chebib, 2017). Post-mortem studies in suicidal individuals have demonstrated epigenetic alterations in the expression and resulting composition of GABA<sub>A</sub>Rs in suicidal individuals (Poulter et al., 2008; Yin et al., 2016), while *in vivo* imaging experiments have revealed functional dysfunctions in GABA<sub>A</sub>Rs in the brain of depressed individuals (Klumpers et al., 2010).

Neurosteroids — endogenous molecules synthesized in the central nervous system from cholesterol — act as positive allosteric modulators of GABA<sub>A</sub>Rs (Baulieu et al., 2001), placing this group of substances in a prominent position regarding the development of novel pharmacotherapies for depression. Extensive research has been conducted in this field for the last 20 years and has recently culminated with the approval of brexanolone, an intravenous formulation of allopregnanolone, as a new strategy for the treatment of severe postpartum depression by the United States Food and Drug Administration (Meltzer-Brody et al., 2018; Scott, 2019). The neurosteroid allopregnanolone (3α,5α-tetrahydroprogesterone, often abbreviated as 3α,5α-THP) presents a particularly high potency of positively modulating both synaptic and extrasynaptic GABA<sub>A</sub>Rs (Carver and Reddy, 2013). Like other neurosteroids, its synthesis from cholesterol begins in the mitochondria with the cleavage of its side-chain, which gives origin to the neurosteroid precursor pregnenolone. In the cytoplasm, the action of the 3β-hydroxysteroid dehydrogenase (HSD) makes the conversion of pregnenolone to the widely distributed steroid hormone progesterone, which can then be metabolized to allopregnanolone by the successive action of two enzymes: 5α-reductase and 3α-HSD (Mellon et al., 2001). Importantly, the synthesis of allopregnanolone is downregulated in depressed individuals, as evidenced by its diminished levels in the cerebrospinal fluid (CSF) (Uzunova et al., 1998) and plasma (Schüle et al., 2006).

A significant portion of the research regarding the antidepressant effects of allopregnanolone has been conducted in experimental animals. More importantly, these preclinical studies allowed the exploration of specific mechanisms of action by which allopregnanolone might exert its antidepressant effects. In addition to detailing its interaction with GABA<sub>A</sub>Rs and to which subunits it binds with higher affinity, many studies provide valuable insights into the mechanisms by which neurogenesis is related to depressive manifestations and to the antidepressant effects of allopregnanolone and other antidepressants, with the brain-derived neurotrophic factor (BDNF) as the main agent (Nin et al., 2011). These studies in animals took advantage of the possibility of measuring or infusing allopregnanolone in key regions of the limbic

system and generated an extensively rich literature on the physiopathological and therapeutic role played by allopregnanolone in depressive-like behaviors across several experimental models of depression.

Taking this rationale into account, this review presents and discusses studies that explore the role of allopregnanolone on depressive-like behaviors in rodents. We examined reports of antidepressant-like effects of exogenous allopregnanolone or its regulation in several animal models of depression. Furthermore, we explore the evidence that links the depression modulating properties of allopregnanolone with neurogenesis, particularly mediated by the neurotrophic protein BDNF.

### 1.1. Brain allopregnanolone levels in animal models of depression

Several animal models of psychiatric disorders have used rodents to study the role of allopregnanolone in emerging depressive-like behaviors. A common strategy to reach this goal has been to induce a depression-like state in laboratory animals and quantify the levels of allopregnanolone in brain regions of interest (i.e., that integrate the neurocircuit known to be involved in the regulation of mood), comparing them to non-intervened controls. These models are based on what is known of the etiological aspects of depression, namely internal susceptibility (genetic construct) and external agents (environmental stressors). Though some models have been generated based on the genetic/heritable aspect of depression, most are based on the induction of a depression-like state through the application of stressors. The successful induction of this depression-like state is frequently confirmed by applying behavioral tests that measure ethological manifestations analogous to depressive symptoms. In this section, we review (Table 1) and discuss the most common models used to these ends and what they reveal about the role of allopregnanolone in the neurobiology of depression.

### 1.2. Forced swim test

Most of the behavioral data that will be presented in the following sections come from the forced swim test (FST), an animal model widely used to detect antidepressant-like activity across different classes of both potential and well-established antidepressant agents. The FST is based on the quantification of the time spent immobile by the rodent while being forced to swim (in rats, 24 h after a previous, longer exposure), which is interpreted as a depressive-like behavior (Detke and

Lucki, 1995; Porsolt et al., 1977). It has excellent predictive validity and reproducibility, as well as a significant translation between the clinical potency and the potency of antidepressants detected in the test (Slattery and Cryan, 2012). Although this model fails in aspects of face validity (e.g.: the detection of acute antidepressant effects of monoamine modulators seldom translate to what is observed in the clinical setting) (Nestler and Hyman, 2010), longer immobile behaviors are seen in animal models with concomitant depression-like states such as diabetes (Gomez and Barros, 2000) and a heritable genetic component has been proposed to influence depressive-like manifestations (Almeida et al., 2018).

Interestingly, the initial stress induced by the forced-swim session is accompanied by an acute increase in brain allopregnanolone that lasts from 10 min to 2 h after a 10-min exposure, as measured in whole or frontal cortex of male rats (Purdy et al., 1991; Vallée et al., 2000). Further experiments corroborated this finding by reporting an increase in the 5 $\alpha$ -reductase enzyme in the prefrontal cortex of male rats (Sánchez et al., 2008). Brain allopregnanolone is known to surge around 30 min after exposure to acute stressors including CO<sub>2</sub> inhalation (Barbaccia et al., 1996), fixation stress (Higashi et al., 2005) and foot-shock (Pisu et al., 2013; Serra et al., 2002), which is likely what drives the observations in the FST. Though the aforementioned findings refer to male rats only, some studies indicate that there seems to be a sex influence, but they point to contradictory conclusions. Pisu et al. (2016) were able to replicate the foot-shock findings in male but not female rats, while Sze et al. (2018) found the opposite, namely a lack of effect in males and an increase in brain allopregnanolone in females after a 2-min FST. Finally, the stress-induced increase in allopregnanolone levels was not replicated in the brains of male mice after exposure to the FST, being that its levels were actually decreased in selective limbic brain areas of these animals (Maldonado-Devincci et al., 2014). Taken together, these results demonstrate that allopregnanolone levels rise in the brain of male rats after exposure to the forced swim test, though this is significantly less certain for female rats or mice. After the initial surge, allopregnanolone levels tend to return to those of unstressed controls, at least in those studies with longer endpoints of up to 2 h (Barbaccia et al., 1996; Purdy et al., 1991), though any further modulations remain unknown.

Thus, the FST is a reliable tool to study the antidepressant-like effects of allopregnanolone in the preclinical setting (as reviewed in Section 1.8). However, it is essential to point out that the FST is not a model of depression *per se*, but rather a model to quantify behaviors that could be considered as being analogous to symptoms of depression in humans, particularly in the context of assessing the effectiveness of antidepressant drug therapies (Nestler and Hyman, 2010). Therefore, even though its application to this end has been successfully established, it is not immediately possible to determine the mechanisms of action by using this model due to some limitations regarding construct validity. Furthermore, several anxiolytic drugs have been long been shown to reduce immobility in the FST (Flugy et al., 1992; Gomez and Barros, 2000) and, since allopregnanolone also presents anxiolytic-like effects (as reviewed in Schüle et al., 2011), it is difficult to distinguish between anxiolytic- and antidepressant-like effects by observing a decrease in immobility.

One other important point to consider is that the findings reported with the FST are mostly obtained in naïve rodents — that is, the animals were not submitted to a long-term protocol aiming to induce a lasting state analogous to depression —, which ultimately does not translate to the target population in humans (i.e., clinically depressed patients). Despite the fact that the detection of antidepressant-like effects in non-depressed-like rodents does not necessarily contradict clinical observations (Serretti et al., 2010), it is reasonable to postulate that the induction of a depression-like state would grant a higher translational value to studies that investigate the role of allopregnanolone on depressive-like behaviors.

### 1.3. Social isolation

One prominent model used to induce a depression-like state is the social isolation paradigm, which is a protocol typically used to model post-traumatic stress disorder (PTSD). In this model, the long-term deprivation of social interaction acts as a powerful stressor that results in a robust and consistently reproducible PTSD-like state in rodents, mainly characterized by the emergence of anxiety-like and aggressive behaviors (Guidotti et al., 2001). In fact, several studies have shown a social isolation-induced increase in aggression against a same-sex intruder in male mice (Pibiri et al., 2006; Pinna et al., 2005, 2003). As recently reviewed by Pinna (2019), the social isolation model may also reflect in some typical depressive-like behaviors due to the overlap between PTSD and depressive disorders. Though a review by Bogdanova et al. (2013) has reported mixed evidence on the effects of social isolation in the FST, it failed to mention contemporary studies that showed a clear immobility-inducing effect by social isolation in rats (Djordjevic et al., 2012; Evans et al., 2012) — which certainly tips the scales in favor of the use of social isolation within a depression-like paradigm. Additionally, social isolation has been shown to induce other depressive-like behaviors in rats, namely a decreased preference for sucrose and an increased ejaculation latency (Wallace et al., 2009).

Importantly, social isolation has also consistently been associated with a significant decrease in allopregnanolone levels in different brain regions of male rats (Evans et al., 2012; Pisu et al., 2016; Serra et al., 2000) and mice (Matsumoto et al., 1999; Nelson and Pinna, 2011; Pibiri et al., 2008; Pinna et al., 2004b, 2005). Allopregnanolone is downregulated in the cerebral cortex (Pisu et al., 2016; Serra et al., 2000) and hippocampus (Evans et al., 2012) of rats, as well as in the olfactory bulb (Nelson and Pinna, 2011; Pibiri et al., 2008; Pinna et al., 2004b, 2005), frontal cortex (Matsumoto et al., 1999; Nelson and Pinna, 2011; Pibiri et al., 2008; Pinna et al., 2004b), amygdala and hippocampus of socially isolated mice, remaining unchanged in the cerebellum and in the striatum (Nelson and Pinna, 2011; Pibiri et al., 2008).

There are significantly fewer studies investigating these parameters in female rodents, but the extant literature is complete enough to demonstrate that their response to social isolation is rather distinct from males. A study by Pinna et al. (2005) specifically compared the effects of social isolation in both sexes, showing that this model failed to increase aggression and to reduce allopregnanolone in the olfactory bulb of females. Interestingly, concomitant daily treatment with testosterone propionate in females resulted in increased aggression and reduced olfactory bulb allopregnanolone levels similar to what was observed in males. These findings in females were later replicated and the same modulatory effect was observed in the frontal cortex (Pibiri et al., 2006). In rats, a more recent study showed that, even though social isolation reduced cerebrocortical allopregnanolone in both sexes, the decrease was greater in males than in females (Pisu et al., 2016). These differences are likely related to the distinct dynamic hormonal profile of females, which is intimately involved with progesterone metabolism and behavioral manifestations related to mood and emotions (reviewed by Frye, 2009).

The downregulation of allopregnanolone in brain areas involved with the corticolimbic system after social isolation strongly suggests that this neurosteroid plays an important role in mood disorders and in the emergence of associated depression-like behaviors. Moreover, it indicates that the fluctuations of other hormones, and thus of other neurosteroids in the brain, may exert complementary mood regulation in rodents.

### 1.4. Chronic unpredictable stress

Another model that stands out because of its long history as a classical animal model of depression is the chronic unpredictable stress paradigm (CUS; also called “chronic mild stress”, “chronic variable stress” and other variations). Originally proposed by Paul Willner in

1987, this model consists of the application of a series of variable, unpredictable stressors for a long period of time (5–9 weeks) that results in a depression-like state characterized mainly by anhedonia-like behaviors (Willner, 2017a). There are reports of decreased preference for sucrose (Guo et al., 2017; Qiu et al., 2017; Zhang et al., 2017) and of increased immobility in the FST (Qiu et al., 2017) in male rats submitted to CUS. Anxiety-like behaviors measured in tests such as the elevated plus-maze and of novelty suppressed feeding were also present in animals submitted to CUS (Guo et al., 2017; Qiu et al., 2017; Xu et al., 2018b; Zhang et al., 2017). Notably, all of these behavioral findings were associated with allopregnanolone downregulation in the hippocampus (Guo et al., 2017; Qiu et al., 2017; Xu et al., 2018b; Zhang et al., 2017), prefrontal cortex (Qiu et al., 2017; Xu et al., 2018b; Zhang et al., 2017) and amygdala (Guo et al., 2017). These reductions are further explained by the downregulation of hippocampal and amygdalar mRNA expression of neurosteroidogenic enzymes of importance to the biosynthesis of brain allopregnanolone, such as the 3 $\alpha$ -HSD, 3 $\beta$ -HSD and 5 $\alpha$ -reductase (Guo et al., 2017).

Though some uncertainty regarding the reproducibility of the CUS model has been frequently raised over the years, a significant portion of this apparent problem might derive from factors such as excessively short exposures (two weeks or less) (Willner, 2017b). In fact, the aforementioned studies used three to four week protocols of unpredictable stressors to observe depressive-like behaviors associated with brain allopregnanolone downregulation. The above findings provide additional neurobiological mechanisms for this model, namely allopregnanolone downregulation in key limbic regions associated to behavioral changes induced by several stressors, and highlight the need to further investigate the role of allopregnanolone and other neurosteroids in this paradigm.

### 1.5. Other rodent models

Reports of changes in brain neurosteroid levels in other animal models of depression date as far back as 2003, when Uzunova and colleagues provided the initial evidence that the rat olfactory bulbectomy modulates brain allopregnanolone levels depending on the brain region and the time after the intervention (Uzunova et al., 2003). Later, a model in which rats were selectively bred for high or low infantile ultrasonic vocalizations after maternal separation showed a line-dependent modulation of brain allopregnanolone that was associated with depression-like behaviors in males and proestrus females (Zimmerberg et al., 2005). Other studies using procedures such as the single prolonged stress (2 h-restraint + 20 min-forced swim + loss of consciousness by ether vapor) (Lee et al., 2018; Su et al., 2019; Xu et al., 2018a), time-dependent sensitization (single sequence of 15 inescapable foot-shocks) (Zhang et al., 2014, 2018) and even streptozotocin-induced type 1 diabetes combined with a high fat diet (Qiu et al., 2016), have consistently demonstrated depression-like states in male rats and decreased allopregnanolone levels in the prefrontal cortex and hippocampus.

All of the studies aggregated in this section demonstrated that there is a consistent decrease in brain allopregnanolone levels in corticolimbic regions associated with a plethora of long term stress-based animal models of psychiatric disorders that induce a depression-like state in rodents. Taking all these behavioral results associated with allopregnanolone fluctuations into account, the next stages in experimental research have been to investigate potential therapies/interventions targeted to increase allopregnanolone in the brain regions of interest in order to elicit antidepressant-like effects.

### 1.6. Antidepressant-like action of allopregnanolone

In the next sections of our review, we gather the evidence that demonstrates the capability of allopregnanolone to decrease depressive-like behaviors in rodents when submitted to preclinical screening tests

used to assess antidepressant activity, such as the FST. We begin by exploring the increase in brain allopregnanolone elicited by certain classical antidepressants believed to exert their effects through a different mechanism: the upregulation of brain neurosteroidogenesis.

### 1.7. Stimulation of allopregnanolone biosynthesis by antidepressants

The selective serotonin reuptake inhibitor (SSRI) fluoxetine has widespread use in medicine and is one of the antidepressant drugs that are most commonly used as a positive control during preclinical experiments. This is because it consistently decreases immobility time by increasing swimming in the FST (as originally described by Detke and Lucki, 1995), which is a parameter related to serotonergic mechanisms. Interestingly, the first evidence of a drug-induced modulation of brain allopregnanolone in the context of depression was provided by Uzunov and colleagues in the following year, and also had fluoxetine as the main comparable substance (Uzunov et al., 1996). In this report, a single injection of fluoxetine or paroxetine increased allopregnanolone levels in corticolimbic brain regions of rats for at least 2 h after treatment. These observations sparked interest regarding the capability of other SSRIs to act as selective brain steroidogenic stimulants (SBSs), a possibility that was further supported by subsequent *in vitro* experiments that demonstrated the increased activity of neurosteroidogenic enzymes associated with fluoxetine, paroxetine and sertraline (Griffin and Mellon, 1999). Furthermore, *in vivo* increases in cerebrocortical allopregnanolone levels induced by numerous SSRIs were later reported after a three-week treatment with paroxetine in male mice (Nechmad et al., 2003) and with fluoxetine, desipramine, sertraline and venlafaxine in olfactory bulbectomized male rats (Uzunova et al., 2004). It is worth mentioning that other classes of drugs have also been reported to increase brain allopregnanolone levels, namely the atypical antipsychotics clozapine and olanzapine (Marx et al., 2006, 2003) and the benzodiazepines midazolam (Qiu et al., 2015) and estazolam (Xu et al., 2018b). On the other hand, a single injection of the tricyclic antidepressant imipramine at behaviorally active doses has consistently been shown not to change brain allopregnanolone levels after 30 min (Pinna et al., 2003, 2004b; Uzunov et al., 1996), probably due to its reported lack of effect on neurosteroidogenic enzymes (Griffin and Mellon, 1999). This is relevant because it is one of the lines of evidence that indicates that not all antidepressants act by this mechanism and that allopregnanolone upregulation is not simply a side effect of monoamine reuptake, but rather a direct effect of SSRIs like fluoxetine. The precise mechanism by which fluoxetine increases brain allopregnanolone levels has not yet been fully determined, but early evidence pointed to the direct activation of the 3 $\alpha$ -HSD enzyme which converts 5 $\alpha$ -dihydroprogesterone into allopregnanolone (Griffin and Mellon, 1999). This finding could not be replicated, however (Trauger et al., 2002), and more recent evidence indicates that, at least in female rats, the inhibition of a steroid microsomal dehydrogenase may consist in a more robust mechanism by avoiding allopregnanolone oxidation to 5 $\alpha$ -dihydroprogesterone (Fry et al., 2014).

Importantly, fluoxetine has been shown to increase brain neurosteroid content at doses remarkably lower than those needed to inhibit serotonin reuptake, as determined by *ex vivo* uptake measurements on brain slices or *in vivo* serotonin detection by microdialysis (Deval et al., 2015; Pinna et al., 2003, 2004b). Thus, these findings granted further support for a direct action of fluoxetine on steroidogenesis, as outlined above. Additionally, they raised the question of whether the administration of fluoxetine at such low doses is capable of eliciting a similar antidepressant-like action by upregulating brain allopregnanolone while being devoid of any significant serotonergic action. Early studies have failed to detect an antidepressant-like effect in the mice FST with low-range doses (1 and 5 mg/kg) (Khisti et al., 2000; Khisti and Chopde, 2000), but a later work achieved this goal in rats using doses as low as 1 and 2 mg/kg (Molina-Hernández et al., 2005). This indicates that a possible antidepressant-like effect of non-SSRI doses of fluoxetine

hovers just over the detectable threshold in the FST, and different species or subtle modifications in the protocol might affect its results.

Given the difficulty generated by such a low and narrow dose window, most of the studies that investigated the antidepressant-like action of allopregnanolone opted to directly administer the neurosteroid instead of upregulating its levels by using drugs that act indirectly and might exert the same target effect by confounding mechanisms.

### 1.8. Exogenous allopregnanolone administration

The seminal studies on the antidepressant-like effect of exogenous allopregnanolone were conducted by Khisti and colleagues in 2000, in which immobility in the FST was reduced 30–60 min after a single intraperitoneal administration in male mice, in doses that ranged between 0.5 and 2 mg/kg (Khisti et al., 2000; Khisti and Chopde, 2000). The same effect was also detected in ovariectomized female mice in a dose range of 0.5–5 mg/kg (Rodríguez-Landa et al., 2007). To the best of our knowledge, only one study reported a lack of behavioral effects after systemic allopregnanolone injection in male rats, in spite of its neurochemical effects (Naert et al., 2007). However, it is important to point out that even though this study evaluated a comprehensive timeframe after treatment, the only dose tested (0.05 mg/kg) is ten times lower than the lowest dose ever reported to elicit antidepressant-like effects after systemic acute treatment. Chronic treatment regimens with subcutaneous allopregnanolone, over several weeks, have also shown antidepressant-like activity in rats, whether in ovariectomized females (Molina-Hernández et al., 2005) or in males (Evans et al., 2012). It must be mentioned that allopregnanolone easily crosses the blood-brain barrier (Hellgren et al., 2014), and the systemic administrations of the neurosteroid allow the assessment of its whole-brain effect. However, a common and more cost-effectively approach has been to infuse microdoses of the neurosteroid directly into the cerebral ventricles, from where it diffuses across the brain with similar distribution and antidepressant-like effects to the systemic injection (Almeida et al., 2018; Khisti et al., 2000; Khisti and Chopde, 2000; Shirayama et al., 2011).

Other studies directed these microinjections to specific areas of the limbic system, with the ultimate goal of assembling a more detailed map of the probable specific brain sites of action where allopregnanolone evokes its antidepressant-like effect. These studies targeted areas where allopregnanolone levels were downregulated in animal models of depression (see Sections 1.3, 1.4 and 1.5) and used the same dose range as in the intracerebroventricular protocols. The brain area that presents the most replicated results is the hippocampus (Nin et al., 2008; Rodríguez-Landa et al., 2009; Shirayama et al., 2011), with the amygdala (Shirayama et al., 2011) and the nucleus accumbens (Molina-Hernández et al., 2005; Nin et al., 2012) contributing as important regions for the antidepressant-like effects in the FST. However, in contrast with the well-reported allopregnanolone downregulation in the prefrontal cortex associated with animal models of depression (see Sections 1.3, 1.4 and 1.5), the few studies that have infused allopregnanolone in this brain area did not detect important behavioral changes in the FST (Almeida et al., 2019; Shirayama et al., 2011). All of these results (compiled in Table 2) support the hypothesis that allopregnanolone, in a similar manner as some neurotransmitters involved with depression, exerts its antidepressant role through pathways associated with the limbic system, probably acting on all the main brain sites responsible for the balance that promotes depressive/anti-depressive regulation.

A few studies have reported behavioral changes following exogenous allopregnanolone administration to rodents submitted to animal models of depression. In mice, a single intraperitoneal injection of allopregnanolone reduced the social isolation-induced aggressive behavior in males and females (Pibiri et al., 2006). Additionally, in socially isolated rats, a subcutaneous insertion of allopregnanolone-containing

pellets normalized immobility time in the FST, both when treatment was started at the onset of isolation or six weeks into the protocol (Evans et al., 2012). More recently, the intracerebroventricular infusion of allopregnanolone has been reported to reduce depressive-like behaviors in a line of rats that were selectively bred to present high immobility in the FST, while having no effect on the line bred to present low immobility in the FST (Almeida et al., 2018). Apart from these studies, researchers have generally preferred to treat depressed-like animals with neurosteroidogenesis-modulating drugs, verifying their behavioral effects, and then quantifying allopregnanolone in brain regions of interest.

## 2. Pharmacological allopregnanolone upregulation: behavioral effects in animal models of depression

SSRIs — specially fluoxetine and norfluoxetine — have been the most frequently used drugs to attempt a reversion of brain allopregnanolone downregulation in animal models of depression. A particularly relevant subset of these studies has tested the effect of a single administration of these drugs at non-SSRI doses (Table 3). In socially isolated male mice, for instance, low doses of fluoxetine have been shown to normalize allopregnanolone levels in the frontal cortex of mice without eliciting the same effect in group-housed animals (Matsumoto et al., 1999). Posterior studies have detailed the stereotypic-specific neurosteroidogenic action of fluoxetine and its active metabolite norfluoxetine in this model, demonstrating that the S-isomers of both drugs — but especially norfluoxetine — showed much higher potency in reducing aggressive behavior (Pinna et al., 2004b) and increasing allopregnanolone levels in the frontal cortex and olfactory bulb (Pinna et al., 2003, 2004b). S-norfluoxetine at similar non-SSRI doses exerted the same effect in female mice treated with testosterone propionate (Nelson and Pinna, 2011), and in male mice when infused directly into the basolateral amygdala (Pibiri et al., 2006). The presence of a depression-like state seems to be important for this neurosteroidogenic effect to take place, since low doses of fluoxetine have been reported not to increase whole brain allopregnanolone in naive male rats (Fry et al., 2014).

Regarding the classic SSRI doses (Table S1), long-term oral treatment with fluoxetine has also successfully restored brain allopregnanolone levels in models of social isolation (Evans et al., 2012), chronic unpredictable stress (Guo et al., 2017), single prolonged stress (Lee et al., 2018), and streptozotocin-induced diabetes combined with high fat diet (Qiu et al., 2016), in regions such as the prefrontal cortex and hippocampus. This normalization was accompanied by antidepressant (Evans et al., 2012; Qiu et al., 2016) and anxiolytic-like (Lee et al., 2018) effects, but the doses applied in these studies were high enough to also inhibit serotonin reuptake. Chronic treatment (10–30 days) with sertraline (15 mg/kg) has also normalized (Qiu et al., 2017; Su et al., 2019; Xu et al., 2018b, 2018a; Zhang et al., 2018) or at least shown a tendency to increase (Zhang et al., 2014) brain allopregnanolone levels in stress-induced animal models of depression while exerting antidepressant-like (Qiu et al., 2017) or anxiolytic-like (Qiu et al., 2017; Su et al., 2019; Xu et al., 2018b, 2018a; Zhang et al., 2018) effects. These reports come mainly in the context of positive controls from studies that demonstrated that some biologically active compounds purified from natural extracts are able to increase brain allopregnanolone (Guo et al., 2017; Lee et al., 2018; Qiu et al., 2017; Su et al., 2019; Xu et al., 2018b), suggesting that the group of SBSSs may be larger than currently estimated (Table S2). However, it is important to point out that these latter findings lack independent replication and their neurosteroidogenic mechanism of action remains even more elusive than that of SSRIs.

A specific molecular target unrelated to SSRIs that has received crescent attention in the past years has been the 18 kDa translocator protein (TSPO), a five transmembrane domain protein that is mainly located in the mitochondria of glial cells (Costa et al., 2012). This

**Table 2**  
Behavioral effects of exogenous allopregnanolone administration in rodents.

Published in	Spe-cies	Sex	Dose(s)	Route	Time before test	Region(s)	Behavioral test (s)	Main findings
Khisti and Chopde (2000)	mice	♂	1 × 0.5–2 mg/kg	i.p.	30 min	● Syst.	● FST	● 0.5; 1; 2: ↓immobility
Khisti et al. (2000)	mice	♂	1 × 0.5–2 μg/mouse	m.i.	15 min	● CV	● FST	● 1; 2: ↓immobility
Molina-Hernández et al. (2005)	mice	♂	1 × 0.5–2 mg/kg	i.p.	30 min	● Syst.	● FST	● 0.5; 1; 2: ↓immobility
Molina-Hernández et al. (2005)	mice	♀	1 × 0.5–2 μg/mouse	m.i.	15 min	● CV	● FST	● 1; 2: ↓immobility
Rodríguez-Landa et al. (2007)	rats	♀	21 × 0.5–2 mg/kg/d	s.c.	30 min	● Syst.	● FST	● 1.5; 2: ↓immobility (↑climbing)
Rodríguez-Landa et al. (2007)	rats	♀ <sub>OVX</sub>	1 × 1–4 μg/rat	m.i.	15 min	● NAcc	● FST	● 3; 4: ↓immobility (↑climbing)
Pibiri et al. (2006)	mice	♂ + ♀	1 × 1.25–5 mg/kg	i.p.	30 min	● Syst.	● FST	● 1; 2; 3: ↓immobility
Naert et al. (2007)	rats	♂	1 × 0.05 mg/kg	i.p.	15–300 min	● Syst.	● RIT	● ALLO (2.5; 5): ↓aggression
Nin et al. (2008)	rats	♂	3 × 1.25–5 μg/rat	m.i.	24, 5, 1 h	● HPC	● FST	● No changes in the FST
Rodríguez-Landa et al. (2009)	rats	♂	1 × 2 μg/rat	m.i.	immedia-tely	● HPC <sub>LS</sub>	● FST	● 2.5: ↓immobility
Shirayama et al. (2011)	rats	♂	1 × 0.1 or 1 μg/rat	m.i.	4 d	● HPC <sub>SF</sub>	● OFT	● HPC <sub>LS</sub> and HPC <sub>D</sub> : ↓immobility
						● HPC <sub>D</sub>		
						● CV	● LHT	● 1: ↓lat./failure to escape
						● HPC <sub>CA3</sub>	● OFT	○ ICV, HPC <sub>CA3</sub> and CeA
						● HPC <sub>DG</sub>		● HPC <sub>DG</sub> , PFC and BLA: no effect
						● PFC		● No changes in the OFT
						● NAcc		
						● BLA		
						● CeA		
Evans et al. (2012)	rats	♂	50 mg released over 50 days	s.c.		● Syst.	● FST	● ↓immobility
							● NSFT	
Nin et al. (2012)	rats	♂	3 × 1.25–5 μg/rat	m.i.	24, 5, 1 h	● NAcc	● FST	● 5: ↓immobility (↑climbing)
							● M-Groom	● 1.25; 5: ↓% correct transitions
Almeida et al. (2018)	rats	♂	3 × 5 μg/rat	m.i.	24, 5, 1 h	● CV	● FST	● ↓immobility (↑swimming)
Almeida et al. (2019)	rats	♂	3 × 1.25–5 μg/rat	m.i.	24, 5, 1 h	● PFC	● FST	● No changes in the FST and M-Groom
							● M-Groom	

Abbreviations and legends: increases (↑); decreases (↓); does not change (≅); ovariectomy (OVX); systemic (syst.); intraperitoneally (i.p.); subcutaneously (s.c.); microinjection (m.i.); hippocampus (HPC); dentate gyrus (DG); dorsal (D); lateral septum (LS); septofimbrial (SF); prefrontal cortex (PFC); central amygdala (CeA); basolateral amygdala (BLA); cerebral ventricle (CV); nucleus accumbens (NAcc); forced swim test (FST); open field test (OFT); resident-intruder test (RIT); learned helplessness test (LHT); novelty-suppressed feeding test (NSFT); microstructured grooming (M-Groom); latency (lat.).

protein is believed to play a major role in the transport of cholesterol to the inner mitochondrial membrane (Schüle et al., 2011), though this has been disputed given that TSPO-knockout mice present normal steroidogenesis, at least in non-cerebral tissue (Morohaku et al., 2014; Tu et al., 2014). What is known is that once cholesterol enters the mitochondria, it can then be converted to neurosteroid precursor pregnenolone by P450<sub>scc</sub>, making its transport a putative rate-limiting step for brain neurosteroidogenesis (Schüle et al., 2011). In fact, lentivirus-induced overexpression of this protein in the dentate gyrus has been shown to increase hippocampal allopregnanolone concentrations in mice (Li et al., 2017) and to normalize its levels in the dentate gyrus of mice exposed to a foot-shock-induced PTSD model (Zhang et al., 2018). These modulations were accompanied by reductions in depressive-like behaviors (Li et al., 2017) and anxiolytic-like effects (Zhang et al., 2018), making TSPO a promising therapeutic target for psychiatric disorders, especially in depression. Because TSPO expression is also increased in damaged neural tissue and is involved with inflammatory responses associated with neurodegenerative disorders (Dupont et al., 2017; McNeela et al., 2018), the specificity of this target must be further investigated and its role in other disorders must be considered as well.

In pharmacological approaches, long-term treatment (18 days–6 weeks) with TSPO agonist YL-IPA08 at a dose of 0.3 mg/kg/day has been shown to normalize brain allopregnanolone levels while abolishing depressive- (Zhang et al., 2017) and anxiety-like behaviors (Zhang et al., 2017, 2014) induced by stress-induced models of depression. Similar protocols using another TSPO ligand, AC-5216, have also reversed depressive- (Qiu et al., 2016; Zhang et al., 2017) and anxiety-like (Zhang et al., 2017) behaviors by increasing allopregnanolone in the prefrontal cortex and hippocampus. TSPO is also a molecular target for benzodiazepines (hence its previous denomination of “peripheral-type benzodiazepine receptor”), explaining why

midazolam, for instance, decreases immobility in the FST while increasing brain allopregnanolone levels (Qiu et al., 2015).

Taken together, all these data suggest that fluoxetine and nor-fluoxetine, in doses which only act via neurosteroidogenic pathways (that is, as SBSSs agents), but also in the classical posology that elicits serotonin reuptake inhibition, exert antiaggression, antidepressant, and anxiolytic-like effects. The aggressive-like induction is a specific indicator of stress and is highly associated with the PTSD-like behaviors induced by social isolation, which can also evoke depressive and anxiety-like states. Also, despite the relatively small number of articles addressing the antidepressant effect of TSPO manipulation, the results seem to be highly replicable and robust (synthesized in Table 3), indicating that pharmacological induction of this neurosteroids synthesis show satisfactory results regarding its antidepressant-like effects in rodents.

### 3. Allopregnanolone and neurotrophic pathways

The protagonism regarding the mechanism of action by which allopregnanolone elicits its antidepressant effects has classically been attributed mainly to the GABAergic system. Like several other similar neuroactive steroids, allopregnanolone acts as a positive allosteric modulator on the main inhibitory receptor of the nervous system, the GABA<sub>A</sub>R (for a more detailed review on this topic, see the paper by Zorumski et al., 2019). Other neurotransmitter systems have also been implicated — though with remarkably less evidence — in that behavioral response, either directly or indirectly. Serotonin, as the main neurotransmitter involved with mood regulation, has been shown to modulate antidepressant allopregnanolone effect (Khisti and Chopde, 2000), as well as dopamine (D’Aquila et al., 2010; Frye et al., 2004), and some neurosteroidogenic enzymes (Espallergues et al., 2012; Khisti and Chopde, 2000; Pinna et al., 2005).

**Table 3**  
Behavioral effects of the pharmacological normalization of brain allopregnanolone in animal models of depression.

Published in	Species	Sex (es)	Model	Treatment(s)		Dose(s) in mg/kg*	Ro-ute	Duration	Behavioral tests		Measu-red after	Brain areas(s)	Main findings	
				Name	Dura-tion				Drug(s)	Drug(s)			Brain allopregnanolone levels	Behavioral changes
<b>Fluoxetine (FLX) and norfluoxetine (NFLX) at selective brain steroidogenic stimulant doses</b>														
Matsumoto et al. (1999)	Mice	♂	SI	2–10 wks	● FLX	0.45; 0.9	i.p.	Single	None		0.5 h	● FC	● FLX: ↑ ( $_{0.9} > \uparrow_{0.45}$ )	N/A
Pinna et al. (2003)	Mice	♂	SI	1 d–8 wks	● R-FLX ● S-FLX ● R-NFLX ● S-NFLX	0.37; 0.45 0.14–0.45 0.27 0.07; 0.27	i.p.	Single	RIT		0.5 h	● OB	● R-FLX (0.45): ↑ ↓aggression ● S-FLX (0.37; 0.45): ↑ ● S-FLX (0.28–1.1): ↑ ↓aggression ● R-NFLX: ≅ ● S-NFLX (0.07; 0.27): ↑ ↓aggression ● S-NFLX (0.53–1): ↓aggression ● S-NFLX (0.09–1): ↓aggression	● R-FLX (0.45–1.1): ↓aggression ● S-FLX (0.28–1.1): ↓aggression ● R-NFLX: ≅ ● S-NFLX (0.53–1): ↓aggression ● S-NFLX (0.09–1): ↓aggression
Pinna et al. (2004b)	Mice	♂	SI	4–6 wks	● R-FLX ● S-FLX ● R-NFLX ● S-NFLX	0.37; 0.6 0.14–0.6 0.04–0.27 0.07–0.27	i.p.	Single	None		0.5 h	● OB ● FC	● R-FLX (0.6): ↑ ● S-FLX (0.37–0.6): ↑ ● R-NFLX: ≅ ● S-NFLX (0.07–0.27): ↑	N/A
Pihiri et al. (2006)	Mice	♀	SI + TP	3 wks	● S-NFLX ● S-NFLX	0.53; 1.1	i.p.	Single	RIT		N.I.	● OB ● FC	● S-NFLX: ↑ ↓aggression	● S-NFLX: ↓aggression
Nelson and Pinna (2011)	Mice	♂	SI	4 wks	● S-NFLX (in BLA)	1.1 µg/mouse	m.i.	Single	RIT		< 1 h	● OB ● FC ● BLA ● HPC ● STR	● S-NFLX: ↑ in BLA and HPC ↓aggression	● S-NFLX: ↓aggression
<b>TSPO modulators</b>														
Zhang et al., 2014b	Rats	♂	TDS	Single event	● YL-IPA08 ● PK11195	0.1–1 1; 3	i.g. i.p.	18 d 18 d	CFCT EPM		1 h 1 h	● PFC	● YL-IPA08 (0.3): ↑ ○ PK11195 → ↑	● YL-IPA08 (0.1–0.3): ↓freezing ● YL-IPA08 (0.1–0.3): ↑open arms time
Qiu et al. (2016)	Rats	♂	DMI	2 wks	● AC-5216 ● PK11195	0.1–1 1; 3	i.g. i.p.	13 d 13 d	EFT SPT NSFT OFT		24 h 24 h	● HPC ● PFC	● AC-5216 (0.3; 1): ↑ in PFC ● AC-5216 (1): ↑ ↑sacrose preference in HPC ○ PK11195 → ↑(○) ● PK11195: ≅ ● AC-5216: ↑ ● YL-IPA08 (0.1; 0.3): ↑	● AC-5216 (0.3; 1): ↓immobility ● AC-5216 (1): ↑sacrose preference ● AC-5216 (1): ↓latency to feed ● No changes in the OFT
Zhang et al. (2017)	Rats	♂	CUS	4 wks	● AC-5216 ● YL-IPA08	0.3 0.1–1	i.g. i.g.	6 wks 6 wks	SPT NSFT OFT		N.I. N.I.	● HPC ● PFC	● AC-5216: ↑ ↑sacrose preference ● AC-5216: ↓latency to feed ● AC-5216: ↑rearrings ● YL-IPA08 (0.1–0.3): ↑ ↑sacrose preference ● YL-IPA08 (0.1–1): ↓latency to feed ● YL-IPA08 (0.1–0.3): ↑rearrings	● AC-5216: ↓latency to feed ● AC-5216: ↑rearrings ● YL-IPA08 (0.1–0.3): ↑sacrose preference ● YL-IPA08 (0.1–1): ↓latency to feed ● YL-IPA08 (0.1–0.3): ↑rearrings

(continued on next page)

Table 3 (continued)

Published in	Species	Sex (es)	Model	Treatment(s)		Dose(s) in mg/kg*	Ro-ute	Duration	Behavioral tests		Measu-red after	Brain areas(s)	Main findings	
				Dura-tion	Drug(s)				Behavio-ral tests	Behavioral changes				
Zhang et al. (2018)	Mice	♂	TDS	Single event	<ul style="list-style-type: none"> <li>• TSPO-LV (in HPC<sub>CPG</sub>)</li> <li>• PK11195</li> </ul>	4 × 10 <sup>8</sup> (TU/μl) 3 m.i. i.g.	30 d	CFCT EPM OFT	N.I.	• DG	Brain allopregnanolone levels	Behavioral changes	<ul style="list-style-type: none"> <li>• TSPO<sub>ox</sub>: ↑</li> <li>○ PK11195 → ↑</li> <li>• ↓freezing (PK11195 → effect)</li> <li>• ↑open arms time (PK11195 → effect)</li> <li>• No changes in the OFT</li> </ul>	

Abbreviations and legends: increases (↑); decreases (↓); does not change (≅); blocks (−); hippocampus (HPC); dentate gyrus (DG); prefrontal cortex (PFC); basolateral amygdala (BLA); striatum (STR); olfactory bulb (OB); fluoxetine (FLX); norfluoxetine (NFLX); testosterone propionate (TP); intraperitoneally (i.p.); intragastric gavage (i.g.); microinjection (m.i.); diabetes mellitus type 1 (DM1); chronic unpredictable stress (CUS); social isolation (SI); time-dependent sensitization (TDS); 18 kDa translocator protein (TSPO); lentivirus (LV); overexpression (OX); contextual fear conditioning test (CFCT); elevated plus maze (EPM); sucrose preference test (SPT); novelty-suppressed feeding test (NSFT); forced swim test (FST); open field test (OFT); resident-intruder test (RIT); not applicable (N/A); not informed (N.I.); \*except if noted otherwise. Unless otherwise specified, changes in allopregnanolone levels refer to all interventions used (model or drugs), doses, or brain areas analyzed in each study.

Going beyond the neurotransmitter systems, animal models of depression induced by chronic stress, such as those explored in Sections 1.3, 1.4 and 1.5, have been shown to modify a distinct aspect of neurobiology, namely the brain neurogenesis. This is evidenced by the reduction of both the production and survival of adult-born hippocampal granule cell neurons in animals submitted to these models. Because the effectiveness of antidepressants is largely dependent on these cells, neurogenesis has been considered an important mechanism by which depressive behaviors are modulated (reviewed by Samuels and Hen, 2011). In fact, hippocampal allopregnanolone downregulation is associated with impaired neurogenesis in the same brain region (Evans et al., 2012). In the same direction, robust evidence has been presented to support a role for neurotrophic agents in the antidepressant effect of allopregnanolone and SBSSs. Here, we gather the data showing that the treatment with allopregnanolone or SBSSs alters neurotrophic protein levels in the limbic system of depressed-like rodents by interacting directly with or in parallel to GABAergic modulation, discussing the participation of neurogenesis in depressive-like states.

### 3.1. BDNF: a key neurotrophic protein involved in the behavioral effects of allopregnanolone

Complex interactions between genetics, hormones, neurotransmitters, and environmental factors are involved in depression. BDNF is a crucial mediator of neuronal plasticity, which regulates synaptic composition, neuronal maturation, neurotransmitter release, survival and excitability in the adult nervous system (Huang and Reichardt, 2001). The pro and mature isoforms of BDNF can be synthesized by and released from neurons, being widely distributed in the limbic system. They bind to the tropomyosin receptor kinase B (TrkB), which has a higher affinity for the mature isoform of the neuropeptide (Nagahara and Tuszynski, 2011). Recently, the BDNF-TrkB signaling has been pointed out as a likely mediator between antidepressant agents and the improvement of depressive symptoms (Björkholm and Monteggia, 2016). Stress and depression have been widely documented to reduce the expression of BDNF in both animal and clinical studies. Two meta-analyses have shown that serum BDNF concentrations are low in untreated depressed patients and normalized by antidepressant treatment, and the greater decrease in symptom alleviation was accompanied by a greater increase in serum BDNF concentrations (Molendijk et al., 2014; Sen et al., 2008).

Such observations are more likely to be seen in women than in men (Huang et al., 2008), but the lack of experimental studies in females makes it difficult to verify if these findings are replicable in the brain. One study found that social isolation reduced BDNF in the cerebral cortex of male but not female rats (Pisu et al., 2016), which points to the opposite direction of clinical findings. In fact, though not assessing BDNF specifically, other studies in rodents that used social isolation have revealed a sex-dependent response in aggression-like behavior and brain allopregnanolone levels (being lower in females), as discussed in Section 1.3. However, studies that took a different approach by knocking out the BDNF gene in mice found pro-depressant effects in females but not males, when matched to wild-type controls (Autry et al., 2009; Monteggia et al., 2007). Given the contradicting findings in the literature, the need for more studies that investigate sex-dependent differences on the role of BDNF on depression becomes even more evident.

In humans, these BDNF modulations become especially apparent in the context of pregnancy, since its serum levels decline considerably throughout pregnancy with a subsequent postpartum increase. Moreover, an inverse relationship between depressive symptoms and serum BDNF during the 3rd trimester (Christian et al., 2016) and postpartum (Gazal et al., 2012) is observed. This supports the role of this neurotrophin in the development of postpartum depression, a very serious complication following delivery that may affect 10–15% of

women within the first 3 months postpartum with important consequences to both mother and child (Christian et al., 2016; Gazal et al., 2012). Due to their apparent superior efficacy, SSRIs are the first line of treatment for severe cases of postpartum depression (Kim et al., 2014).

As reviewed by Nin and colleagues in 2011, “the pharmacological actions of SSRIs are induced by their ability to act as SBSSs, which suggests a novel and more selective mechanism for the behavioral action of this class of drugs”. In fact, this review summarizes the association of depression and decreased cerebral and systemic BDNF, and also that SBSSs succeed to reverse these BDNF decreased levels (Nin et al., 2011). In a more recent review, Kojima et al. (2019) offered a possible explanation for the decreased BDNF expression in patients with major depressive disorder and in animal models of depression. Considering that BDNF expression is controlled by neuronal activity, low BDNF pro-peptide levels in the CSF may be the result of lower neuronal activity in the brain of depressed individuals. In fact, there seems to be an important connection between BDNF (both in its pro and mature isoforms) and GABAergic activity, though the specific mechanisms by which this interaction takes place are still being elucidated. Some evidence points to a net excitatory effect in the superior colliculus by postsynaptic inhibition of the GABAergic currents (Henneberger et al., 2002), but since this is not seen in the visual cortex (Abidin et al., 2008) or amygdala (Meis et al., 2019), it seems to be a region-dependent effect. In the hippocampus, BDNF is thought to increase cell surface expression of GABA<sub>A</sub>Rs by TrkB activation-induced inhibition of receptor endocytosis, enhancing GABAergic inhibition (Porcher et al., 2018).

Several studies have demonstrated a general downregulation of BDNF in the hippocampus and frontal cortex in stress-based animal models of depression (Phillips, 2017). As already discussed in this review, such animal models have been shown to decrease allopregnanolone levels in these and other brain areas relevant to the neurobiology of depression (see Sections 1.3, 1.4 and 1.5). In the social isolation protocol, for instance, the reduction in cerebrocortical allopregnanolone is accompanied by decreased hippocampal BDNF in male rats, though not in females (Pisu et al., 2016). Similar effects on BDNF have been observed after exposure to CUS (Rudyk et al., 2019), with a greater magnitude in those animals that present more accentuated depressive-like behaviors (Tornese et al., 2019). This stress-induced downregulation appears to have long-lasting effects since hippocampal BDNF is decreased until seven days after a single prolonged stress protocol (Lee et al., 2018). Additionally, long-term treatment with allopregnanolone (Evans et al., 2012), fluoxetine (Evans et al., 2012; Lee et al., 2018), or other potential SBSSs (Lee et al., 2018) restores the low hippocampal BDNF levels back to normal.

Indeed, the hippocampus appears to be the main region involved with the neurotrophic regulation of allopregnanolone. When the pregnane xenobiotic receptor (PXR) — a protein involved in cholesterol metabolism — is knocked down in rats, a downregulation in hippocampal allopregnanolone and in BDNF is observed, suggesting that PXR may influence the allopregnanolone synthesis by neuroplasticity mechanisms (Frye et al., 2014). Conversely, BDNF levels in that region are increased 3 h following a single low-dose of allopregnanolone i.p. administration (Naert et al., 2007). Moreover, 1 h after sub-acute intraprefrontal cortex infusion, it is increased both in the left and right hippocampus, with a tendency to be higher in the right hemisphere (Almeida et al., 2019). Interestingly, these rapid hippocampal BDNF regulations were observed even in the absence of an associated antidepressant-like effect in the FST.

There is some evidence indicating that the hippocampus is not the only relevant brain region implicated in the BDNF mediation of antidepressant effects. The prefrontal cortex, for instance, has been associated with BDNF reduction after depressant manipulations (Lee et al., 2018; Zhang et al., 2017) and the infusion of allopregnanolone in this area increases BDNF mRNA expression in the left hemisphere of the same region (Almeida et al., 2019). However, given that intra-prefrontal cortex allopregnanolone infusion is without antidepressant-like

effects in rats (Almeida et al., 2019; Shirayama et al., 2011), the role of BDNF in this region is significantly less clear. It is possible that these frontal BDNF alterations in depressed animals are consequences of hippocampal effects, and that local (frontal) allopregnanolone-induced BDNF increases play no role in its antidepressant effect. However, the lack of studies investigating the direct infusion of BDNF in the prefrontal cortex renders these ideas merely speculative.

Nevertheless, BDNF is not the only protein that is likely involved with depressive-like states and neurogenesis in brain limbic areas. Other growth factors participate in cell proliferation, migration, and differentiation, especially in the nervous system. Besides BDNF, some other neurotrophic proteins have been associated with depression and the antidepressant effect of classical antidepressants, especially the nerve growth factor (NGF) (reviewed by Mondal and Fatima, 2019). To our knowledge, there is a single *in vitro* experiment that demonstrated, after exposure to moderate concentrations of allopregnanolone, a decrease in the toxicity of NGF-treated cells (Afrazi et al., 2014). Another neurotrophic protein studied was reelin, which was proposed by Pinna and colleagues as another potential neurogenic protein involved with neurosteroid behavioral attenuation (Pinna et al., 2004a). In that paper, it is shown that there is an increase in aggression in male and female mice associated with a decrease of brain allopregnanolone, and this behavior is reversed with concomitant reelin modulation. Furthermore, in socially isolated male mice, aggression can be prevented by treatment with L-methionine, which has also been shown to decrease reelin (Pinna et al., 2004a).

Moreover, other markers of neurogenesis have provided evidence concerning allopregnanolone's role in neurotropy. The changes in hippocampal allopregnanolone induced by time-dependent sensitization and TSPO overexpression in the dentate gyrus — discussed in Section 2 — were directly associated with the proliferation of progenitor cells as shown by bromodeoxyuridine immunohistochemistry (Zhang et al., 2018). This effect is robust enough to be also observed in a neurodegenerative model induced by chronic treatment with lipopolysaccharide, where an increase in newborn neurons by TSPO overexpression is additionally reported (Wang et al., 2016). The administration of exogenous allopregnanolone has also been shown to restore cell proliferation and rescue cell survival in the subgranular zone of the dentate gyrus after social isolation (Evans et al., 2012), probably through its BDNF-mediated neurogenic effects. These findings indicate that this particular structure, the dentate gyrus, is probably the main functional area within the hippocampus responsible for the neurogenesis mediated by allopregnanolone. And, in addition to influencing neurogenesis, allopregnanolone apparently also inhibits neurodegeneration by suppressing extracellular signal-regulated kinases (ERKs) phosphorylation *in vitro* (Mendell et al., 2018). On the other hand, chronic exposure to exogenous allopregnanolone may evoke the opposite effect, since a regimen of three times/week subcutaneous injection has been shown to decrease recruitment of hippocampal progenitor cells — though one injection/week did increase neurogenesis (Chen et al., 2011). This, in association with the observation that long-term continuous allopregnanolone administration leads to memory decline and hippocampus shrinkage (Bengtsson et al., 2016), demonstrates that the effects of allopregnanolone on neurogenesis is likely dependent on treatment duration and frequency.

All these data suggest that BDNF participates as an important player in the antidepressant effect induced by allopregnanolone, and that its manipulation arises as a promising alternative for the pharmacological approach of depression. In addition, the papers reviewed suggest a wide field to be explored regarding the relationship between allopregnanolone and other neurotrophic proteins, regarding their role in the neurotrophic antidepressant-like effect.

### 3.2. Environmental interventions to increase neurotropy: what role do neurosteroids play?

Because neurogenesis is a process that is intimately linked to a wide array of external factors, animal models of depression represent only a small fraction of the environmental conditions that importantly modify this aspect of brain biology. The input of adequate or inadequate stimuli, particularly during the developmental phases in life, may significantly contribute to a higher or lower pattern of neurogenesis, respectively. One example is maternal care, a complex set of nursing actions that, if executed poorly, may reflect in neurochemical and behavioral deficits in adulthood (Nephew and Murgatroyd, 2013). In fact, one of the conditions that may result in poor maternal care (mainly characterized by grooming and licking of the pups) is the early age social isolation of the mothers, which is also associated with low circulating allopregnanolone levels (Pisu et al., 2017). This factor exerts an important effect on the dams since rats from low licking/grooming dams present more anxiety-like behaviors and lower hippocampal allopregnanolone levels in adulthood (Borrow and Cameron, 2017). An interesting observation is that these rats were compared to animals from high licking/grooming dams, which presented comparatively higher brain allopregnanolone levels. Thus, it suggests that “positive” life experiences might also exert an effect in neurosteroidogenesis, perhaps mediated by the action of neurotrophic agents.

One strategy to model positive stimuli is to expose the animals — preferably at a young age, generally just after weaning — to an enriched environment. In the laboratory setting, this means to provide a richer housing condition that normally focuses on three main pillars: greater social interaction, diversified sensory input, and incentive to voluntary exercise (van Praag et al., 2000). Environmental enrichment has been associated with neurotrophic changes in the brain, which is supported by a recently published systematic review of animal studies that demonstrated a robust neurogenic effect associated with this paradigm, having BDNF as one of its main regulating agents (Barros et al., 2019). Thus, this paradigm becomes a provocative possibility to investigate the neurogenesis-related upregulation in brain steroid synthesis. Munetsuna et al. (2011) provided the first evidence of modulation in brain steroidogenesis by environmental enrichment in a comprehensive experiment that quantified the hippocampal mRNA expression of 19 different enzymes involved with brain steroid metabolism. They showed that male rats reared in an enriched environment for eight weeks presented a higher expression of the neurosteroidogenic enzymes 5 $\alpha$ -reductase type 1 and 3 $\alpha$ -HSD when compared to standard housing-reared animals. Of particular note, these two enzymes are the main ones involved in allopregnanolone biosynthesis, strongly suggesting its participation in the neuroprotective effects induced by this paradigm. In aged females, environmental enrichment also increased these and other neurosteroidogenic enzymes in the hippocampus (Rossetti et al., 2015). A recent experiment studied the sensory and motor aspects of environmental enrichment separately in a 10-day protocol, showing similar results in young female adults with a much more modest effect in aged rats (Rossetti et al., 2019). In males, four weeks of environmental enrichment reverses the deficits in neuronal plasticity induced by previous social isolation for the same amount of time by restoring hippocampal BDNF, NGF and activity-regulated cytoskeletal associated protein levels, as well as dendritic spine density and other markers of neurotropy (Biggio et al., 2019). Also, some studies have reported an antidepressant-like effect in the FST elicited by environmental enrichment in male rats (Ashokan et al., 2018; Possamai et al., 2015).

Similar neurotrophic changes have been associated with the emergence of resilient phenotypes against chronic stress, preventing the establishment of a depression-like state. In this case, these changes were induced by knocking out a proapoptotic gene (Bax) in mice, which increased the survival of hippocampal cells (Anacker et al., 2018). A resilient phenotype may also be induced by environmental enrichment,

which prevents the emergence of depressive-like behaviors induced by social defeat stress, presumably by also increasing hippocampal neurogenesis (Lehmann and Herkenham, 2011). However, this resilience has not yet been experimentally linked to brain allopregnanolone levels, though this hypothesis has been raised by Biggio et al. (2014). Other studies have attempted to generate resilient rats by selective breeding rats that presented high or low traits related to depression. A study conducted by Frye's research group that bred rats with high or low rates of infantile ultrasonic vocalization (USV) after maternal separation found that the low USV line presented higher brain allopregnanolone levels associated with lower depressive-like behaviors, which suggests an associated resilient profile (Zimmerberg et al., 2005). More recently, we bred rats in our laboratory that presented high or low immobility in the FST and showed that allopregnanolone was only able to reduce immobility in the high immobility line (Almeida et al., 2018).

This limited but important assortment of data suggests that neurosteroids play a crucial role on environmental stimulus during early ages in animals. It remains to be established whether the stress resilience achieved in these studies is dependent on the neuroprotective effects of allopregnanolone, which — if proven true — will broaden the importance of this neurosteroid in the neurobiology of depression.

## 4. Conclusion

All of the aforementioned evidence discussed in this review demonstrates that allopregnanolone plays a major role in depressive-like manifestations in humans and in animal models. The systemic downregulation of allopregnanolone seen in depressed individuals is well-reproduced across several animal models of depression, which provide an additional level of detail regarding its brain regulation. In depressed-like animals, allopregnanolone levels are consistently downregulated in areas of the corticolimbic system that are responsible for mood regulation. Moreover, its infusion in these areas exerts antidepressant-like effects, which evidences its importance in the neurobiology of depression. These preclinical observations led to the development of a formulation fit for intravenous infusions in humans, brexanolone, that demonstrated efficacy for the treatment of postpartum depression and is currently approved for clinical use.

Also, the amelioration of symptoms observed after treatment with widely prescribed antidepressants, particularly SSRIs, is at least partially due to the capacity of these substances to increase brain allopregnanolone content, as largely demonstrated in animal studies. The main drugs that upregulate allopregnanolone levels are SSRIs, which present this neurosteroidogenic property even in lower non-serotonergic doses, which are known to exert an SBSS action. This drug-induced upregulation of allopregnanolone reduces depressive-like behaviors in models such as the FST, which is also achieved with other agents that increase brain neurosteroidogenesis levels by different mechanisms.

Furthermore, among the varied mechanisms by which allopregnanolone might exert its antidepressant effects, the increase in hippocampal neurogenesis by the upregulation of neurotrophic proteins is proving to be a relevant pathway for this antidepressant action, giving origin to a crescent and vibrant field of research. This rationale is associated to the fact that hippocampal neurogenesis is lower in depressed-like animals, and reversed predominantly by increases in BDNF in antidepressant-treated animals. There is plenty of evidence pointing to the role of altered GABAergic function and of altered BDNF in major depressive disorder and in allopregnanolone effects. It is still needed to understand if and how these two mechanisms might be related to the quick, effective and lasting antidepressant effects of neurosteroid antidepressants such as brexanolone. Is one of these mechanisms more important for the clinical effects of brexanolone than the other, or is there a synergism or potentiation between the GABAergic and neurotrophic systems that better explain the effects seen in the clinical setting?

An interplay between allopregnanolone's effects on GABA and on neurogenesis might bring a dual response that has to be investigated regarding brexanolone's rapid and long lasting clinical effects. Also, one may assume that the increase in neurosteroidogenesis by interventions such as the environmental enrichment points to a mechanism through which allopregnanolone is involved with stress resilience. Future studies should further investigate if and how allopregnanolone is able to improve resilience and whether genetic factors play a significant role in this particular pathway of neuroprotection. With the very recent authorization for the use of brexanolone for the treatment of postpartum depression, it becomes evident that allopregnanolone and other neurosteroidogenic agents may be an important tool for the treatment of affective disorders, and may prove to be effective for the treatment of major depression disorder and bipolar disorders in areas where other, more classical antidepressants have failed.

### CRedit authorship contribution statement

**Felipe Borges Almeida:** Conceptualization, Investigation, Writing - original draft. **Maurício Schüller Nin:** Conceptualization, Investigation, Writing - review & editing. **Helena Maria Tannhauser Barros:** Conceptualization, Writing - review & editing, Supervision.

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### Appendix A. Supplementary data

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