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## Developmental and adult stress: effects of steroids and neurosteroids

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### ABSTRACT

In humans, exposure to early life adversity has profound implications for susceptibility to developing neuropsychiatric disorders later in life. Studies in rodents have shown that stress experienced during early postnatal life can have lasting effects on brain development. Glucocorticoids and sex steroids are produced in endocrine glands and the brain from cholesterol; these molecules bind to nuclear and membrane-associated steroid receptors. Unlike other steroids that can also be made in the brain, neurosteroids bind specifically to neurotransmitter receptors, not steroid receptors. The relationships among steroids, neurosteroids, and stress are multifaceted and not yet fully understood. However, studies demonstrating altered levels of progestogens, androgens, estrogens, glucocorticoids, and their neuroactive metabolites in both developmental and adult stress paradigms strongly suggest that these molecules may be important players in stress effects on brain circuits and behavior. In this review, we discuss the influence of developmental and adult stress on various components of the brain, including neurons, glia, and perineuronal nets, with a focus on sex steroids and neurosteroids. Gaining an enhanced understanding of how early adversity impacts the intricate systems of brain steroid and neurosteroid regulation could prove instrumental in identifying novel therapeutic targets for stress-related conditions.

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### Introduction

The word “hormone” was first used over 100 years ago to describe “the chemical messengers which, speeding from cell to cell along the bloodstream, may coordinate the activities and growth of different parts of the body” (Starling, 1905). Originating from the Greek root *hormon*, which means “to arouse or excite,” the term was coined to describe secretin, a substance derived from the digestive system. As more molecules that are produced in the periphery, released into the bloodstream, and act on distant targets were discovered and characterized, the term hormone became generally accepted by the scientific community. Hormones can be roughly divided into three major biochemical classes: (1) steroids; (2) peptides; and (3) amino acid derivatives (Tata, 2005). This review will focus only on the first in this list – steroids.

Many of the molecules originally designated as hormones can be produced in non-endocrine tissue where they exert local action. This is common in the brain where molecules originally designated as hormones are produced by neurons and glia, and have paracrine, autocrine, and intracrine functions. This means they can act on cells nearby the synthesizing cell, act on the synthesizing cell itself, or act within the synthesizing cell, respectively (Rubinow, 2018; Saldanha et al., 2011). Despite compelling evidence for brain synthesis and non-endocrine action of these molecules, the widespread adoption of the term hormone to describe molecules that

have broader mechanisms of action has led to common misunderstandings and obstacles to progress in the field. This is particularly true for some steroid molecules that were originally discovered as originating from the gonads (progestogens, estrogens, androgens) and adrenals (corticosteroids). These molecules were first characterized for their roles in reproductive function and stress responses respectively. It is now abundantly clear that progestogens, estrogens, androgens, and glucocorticoids can be made in the brain where they act on steroid receptors locally (Lloyd-Evans & Waller-Evans, 2020), or in some cases are further metabolized into neurosteroids that can serve as allosteric modulators of neurotransmitter receptors (Wang, 2011). Nonetheless, common misperceptions exist due to the original discovery and characterization of these molecules as hormones. For example, estradiol is widely considered to be a “female sex hormone” due to its peripheral actions, but it is present in much higher concentrations in the brains of adult males than females (Hojo & Kawato, 2018). Furthermore, molecules originally associated with female reproduction, such as progesterone, are increased in the periphery and brains of both males and females in response to stress (Shors et al., 2001; Romeo et al., 2007; Laham et al., 2022).

The broad regional synthesis of steroids and their multiple mechanisms of action have complicated attempts to understand their roles in stress effects, particularly in the context of sex differences. This review attempts to integrate information

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about peripheral and brain concentrations and actions of progestogens, androgens, estrogens, and glucocorticoids, and their metabolites (Table 1), in the context of early life stress effects on neurons, glia, and the extracellular matrix in the brain. The review focuses primarily on brain regions that have been implicated in stress effects and cognitive/emotional processing, including the hippocampus (HIP), basolateral amygdala (BLA), and prefrontal cortex (PFC). To set the stage for this endeavor, we start with a brief description of steroid synthesis in the periphery and brain.

### Sex steroid and glucocorticoid synthesis begins with cholesterol

Progestogens, estrogens, androgens, and corticosteroids are made from cholesterol. Cholesterol is produced primarily in the body with a lower percentage coming from the diet (Miller & Bose, 2011; Rone et al., 2009). Synthesis of these steroids begins in the mitochondria where cholesterol is transported from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) by a lipid binding protein called steroidogenic acute regulatory protein (StAR) (Miller & Bose, 2011) (Figure 1). Another downstream protein to StAR, mitochondrial translocator protein (TSPO), is involved in transporting cholesterol from OMM to IMM (Papadopoulos et al., 1997). The mitochondria contain important enzymes for steroid synthesis, including P450 side-chain cleavage (P450 scc) enzymes (Omura, 2006). The first enzymatic step occurs in the IMM where cholesterol is converted to pregnenolone by the catalyzing action of a P450 scc enzyme (Koritz & Kumar, 1970; Manna et al., 2013; Stocco et al., 2005; Stone & Hechter, 1955).

Pregnenolone can serve as the precursor to many different steroids that have hormonal action, including progestogens, estrogens, androgens, and glucocorticoids (Baulieu et al., 2001) (Figure 2). The presence of relevant enzymes is a main determining factor of whether tissue that generates pregnenolone synthesizes bioactive amounts of these other substances. On its own, pregnenolone does not exert influence over sex steroid receptors, but it does have neurosteroid properties (Vallée et al., 2014) (Figure 2).

### Progestogens

In the mitochondria, pregnenolone can be oxidized into progesterone (P4), the main progestogen, by the enzyme 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD) (Simard et al., 2005) (Figure 1). Once synthesized, P4 diffuses across the OMM to the cytosol where it can bind to intracellular nuclear progesterone receptors (PRs), diffuse across the cell membrane and bind with membrane-associated PRs on other cells, or enter nearby cells to exert its action on nuclear PRs. P4 can also enter the endoplasmic reticulum where, depending on the presence of specific enzymes, it can be converted into other metabolites, some of which also bind to PRs, such as 5 $\alpha$ -dihydroprogesterone (DHP) (Eicheler et al., 1994; Guennoun, 2020). It should be noted that DHP also binds to GABA<sub>A</sub> receptors so it works as a steroid and neurosteroid (Guennoun, 2020).

### Androgens

An alternative biochemical path for progesterone is oxidation to 17-OH progesterone and then to the androgen androstenedione by other cytochrome P450 enzymes in the endoplasmic reticulum (Fevold et al., 1989; Giatti et al., 2020). Androstenedione can be converted to testosterone by the enzyme 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD).

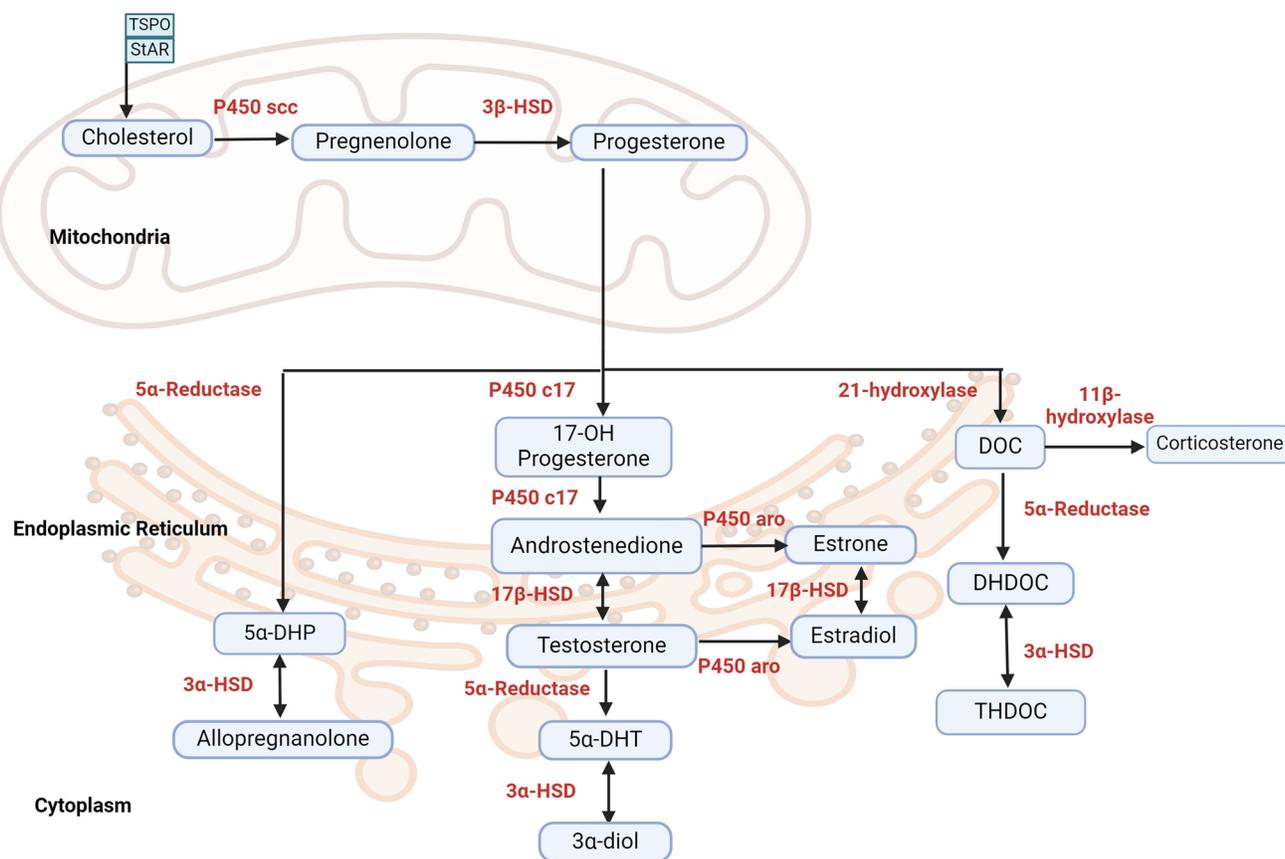
Testosterone is reduced to 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT) by the action of 5 $\alpha$ -reductase (Jin & Penning, 2001). Androstenedione, testosterone and DHT can bind to either nuclear androgen receptors (ARs), which serve as transcription factors and alter gene expression, or membrane-associated androgen receptors, which activate G proteins and alter second messenger cascades (Hiipakka & Liao, 1998; Zucker et al., 1996).

### Estrogens

Progesterone-derived androstenedione can be converted to the estrogen estrone by the enzyme P450 aro and then to estradiol, the most prevalent estrogen in mammals, by the enzyme 17 $\beta$ -HSD (Osawa et al., 1993). Estradiol can also be synthesized by the aromatization of testosterone also through P450 aro (MacLusky et al., 1994). Both of these biochemical

**Table 1.** Chemical Names, Common Names, and Abbreviations for Neuroactive Derivatives of Sex Steroids and Glucocorticoids.

| Chemical name   | Common name  | Abbreviation          |
|---|--|-----------------------|
| Pregn-4-ene-3,20-dione                                | Progesterone, Pregnenedione  | P4                    |
| 17 $\beta$ - estradiol                                | Estradiol  | E2                    |
| 17 $\beta$ - hydroxy-4-androsten-3-one                | Testosterone   | T                     |
| 11 $\beta$ , 21-dihydroxy-4-pregnene- 3,20-dione      | Corticosterone, 17-Deoxycortisol                                       | CORT                  |
| 3 $\alpha$ -Hydroxy-5 $\alpha$ -pregnan-20-one        | Allopregnanolone   | ALLO                  |
| 3 $\beta$ -Hydroxypreg-5-en-20-one                    | Pregnenolone   | P5                    |
| 3 $\alpha$ , 21-Dihydroxy-5 $\alpha$ -pregnan-20- one | Tetrahydrodeoxycorticosterone  | THDOC                 |
| 5 $\alpha$ -androstan-3 $\alpha$ , 17 $\beta$ -diol   | Androstanediol   | 3 $\alpha$ -diol      |
| 5 $\alpha$ -pregnane-3, 20-dione                      | 5 $\alpha$ -Dihydroprogesterone  | 5 $\alpha$ -DHP       |
| 21-hydroxy-5 $\alpha$ -pregnan-20-one                 | 5 $\alpha$ -Dihydrodeoxycorticosterone                                 | DHDOC                 |
| 21-Hydroxy-4- pregnene-3,20- dione                    | Deoxycorticosterone,<br>11-Deoxycorticosterone, 21-hydroxyprogesterone | DOC                   |
| 3-hydroxyestra-1,3,5 (10)-trien- 17-one               | Estrone  | E1                    |
| 17 $\alpha$ -hydroxyprogesterone                      | 17-Hydroxyprogesterone   | 17-OH<br>Progesterone |



**Figure 1.** Subcellular Compartments for Steroid/Neurosteroid Synthesis. Diagram showing that steroid/neurosteroid synthesis occurs in the mitochondria and endoplasmic reticulum.

Cholesterol enters the mitochondria facilitated by StAR and TSP0 protein where it is cleaved into pregnenolone by enzyme P450scc. Pregnenolone is converted to progesterone (P4) by 3β-HSD. In the endoplasmic reticulum, progesterone is converted to testosterone by a two-step pathway. 17-hydroxy progesterone and androstenedione catalyzed by P450c17 and 17β-HSD respectively. T is reduced to 5α-DHT and then 3α-diol. P450 aromatase enzyme (P450 aro) reduces androstenedione and T into estrone and estradiol respectively. In the cytoplasm, progesterone is also catalyzed into 5α-DHP and then into allopregnanolone by 5α-reductase and 3α-HSD). Estrone is reversibly converted to estradiol by 17β-HSD. Progesterone is converted into DOC by 21-hydroxylase which undergoes two subsequent reduction steps by 5α-reductase and 3α-HSD to form THDOC. DOC can also be converted into corticosterone by 11β-hydroxylase. Enzymes are denoted in red, steroids and neurosteroids in blue boxes. The arrows and directional arrows indicate irreversible and reversible reactions respectively. Abbreviations; StAR; steroidogenic acute regulatory protein; TSP0, translocator protein; p450scc, P450 side chain cleavage; P4, progesterone; HSD, hydroxysteroid dehydrogenase; 17-OH progesterone, 17-hydroxyprogesterone; 5α-DHT, 5α-dihydrotestosterone; 5α-DHP, 5α-dihydroprogesterone; DOC, deoxycorticosterone; THDOC, tetrahydrodeoxycorticosterone

reactions occur in the endoplasmic reticulum. Estrogen receptors (ERs) include those that are nuclear, whose activation alters gene expression, or membrane-associated, whose activation alters G protein signaling cascades (McEwen, 2002).

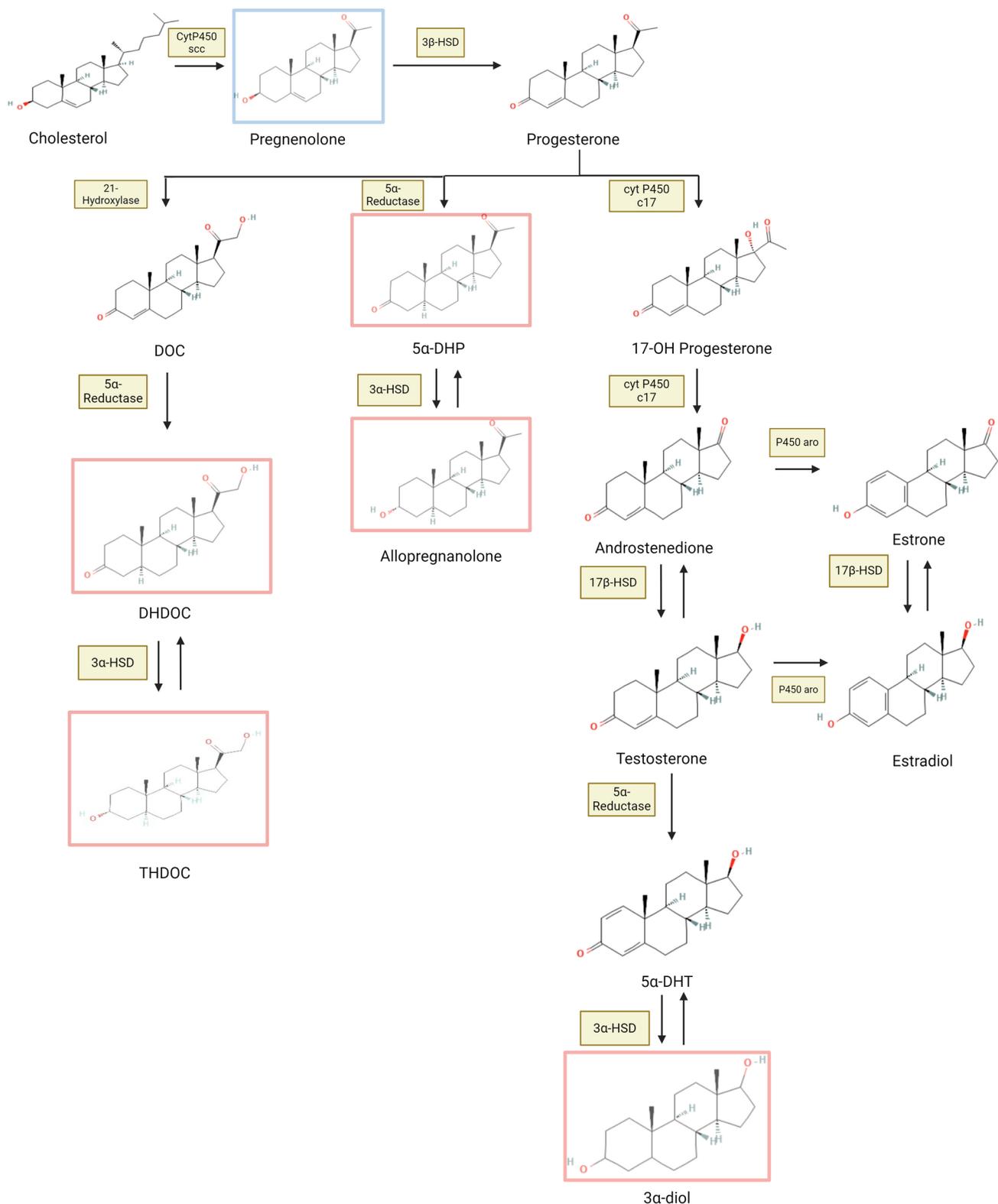
### Glucocorticoids

An additional biochemical pathway for progesterone is its conversion to deoxycorticosterone (DOC) by the enzyme 21-hydroxylase. Next, DOC can be converted to corticosterone, the main rodent glucocorticoid, by the action of the enzyme 11β-hydroxylase. In primates and many other nonrodent mammals, the enzyme 17α-hydroxylase, which rodents lack (Raff, 2016), enables the production of 17α-hydroxyprogesterone, a substrate that is converted to cortisol by 21-hydroxylase and 11β-hydroxylase (Porcu et al., 2016; Van Belle, 2017). The steroids involved in both of these pathways have been shown to bind to both nuclear and membrane-associated glucocorticoid receptors (GRs) with the former altering gene expression and the latter activating G-protein coupled signaling cascades (Heitzer et al., 2007).

### Determining factors of sex steroid and glucocorticoid synthesis

There are many similarities among the biochemical pathways involved in the synthesis of progestogens, androgens, estrogens, and glucocorticoids. First, all of the relevant steroids derive from the same precursor molecule, pregnenolone (Figure 2). Second, androgens, estrogens, and glucocorticoids all derive from progesterone. Third, many of the biochemical pathways use the same enzymes, with different products arising from the action on different substrates. For example, 17β-HSD can convert estrone to estradiol as well as androstenedione to testosterone (Fevold et al., 1989).

Given the shared biochemical pathways, it is not surprising that most tissues that synthesize one type of these steroids also synthesize the others, albeit at different concentrations. For example, the adrenal glands, the testes, the ovaries, and the brain all produce progestogens, androgens, estrogens, and glucocorticoids (Dufau et al., 1971; Holst et al., 2004; Nelson & Bulun, 2001; Rosol et al., 2001). Differential concentrations of these steroids in specific tissues depend on both



**Figure 2.** Chemical Structures of Neurosteroid Synthesis. Neurosteroids are closely related structurally, which allows many to serve as ligands for the same receptors. Neurosteroids binding to GABA<sub>A</sub> receptors and endocannabinoid receptors are surrounded by red and blue boxes respectively. Abbreviations: HSD, hydroxysteroid dehydrogenase; P450 scc, P450 side chain cleavage enzyme; P450 aro, P450 aromatase; DOC, deoxycorticosterone; DHDOC, dihydrodeoxycorticosterone; THDOC, tetrahydrodeoxycorticosterone; 5 $\alpha$ -DHP, 5 $\alpha$ - dihydroprogesterone; 17-OH progesterone, 17-hydroxyprogesterone; 5 $\alpha$ -DHT, 5 $\alpha$ - dihydrotestosterone.

the expression of specific enzymes as well as on the concentration of specific substrates.

Not surprisingly given their shared biochemical pathways, these molecules also have similar molecular structures (Figure

2). The molecular similarity of many of these steroids confers cross-steroid receptor ligand status to several of them. For example, progesterone binds to PRs with highest affinity, but it also binds to GRs (Beato et al., 1995; Kontula et al., 1983),

and testosterone binds with highest affinity to ARs, but it also binds to PRs and ERs (Chang et al., 1995; Rochefort & Garcia, 1976). These features make it difficult to find agonists and antagonists of these receptors that are specific. For example, mifepristone, a well-known PR antagonist, is also a potent GR antagonist (Im & Appleman, 2010). In addition to the complex interactions of sex steroids and glucocorticoids with their receptors, these molecules exert action in the brain through their conversion to neurosteroids.

### Sex steroids and glucocorticoids can be converted to neurosteroids

Neurosteroids are steroids synthesized in the central nervous system by both neurons and glial cells (Baulieu, et al., 2001). Neurosteroids are different from sex steroids and glucocorticoids in that they exert their effects by binding to neurotransmitter receptors as opposed to nuclear or membrane bound-steroid receptors (Mellon & Griffin, 2002; Penning et al., 2004; Schverer et al., 2018). In addition to serving as a precursor to progesterone, androgens, estrogens, and glucocorticoids, pregnenolone itself can act as a neurosteroid by binding to endocannabinoid receptors where it acts as an allosteric inhibitor (Vallée et al., 2014). Progesterone, androgens, and glucocorticoids can also all be converted to neurosteroids that bind to GABA receptors (Reddy & Jian, 2010).

As mentioned above, progesterone can be metabolized to DHP by the action of 5 $\alpha$ -reductase. DHP is then converted to 3 $\alpha$ , 5 $\alpha$  tetrahydroprogesterone (3 $\alpha$ , 5 $\alpha$ -THP), or allopregnanolone, in the presence of the enzyme 3 $\alpha$ -HSD (Penning et al., 2004). When acting on testosterone, 5 $\alpha$ -reductase produces DHT, which is further converted to the neurosteroid 3 $\alpha$ -androstenediol (3 $\alpha$ -diol) in the presence of 3 $\alpha$ -HSD (Jin & Penning, 2001). The glucocorticoid deoxycorticosterone is reduced to dihydrodeoxycorticosterone (DHDOC) by the action of 5 $\alpha$ -reductase and then the enzyme 3 $\alpha$ -HSD converts it to the neurosteroid tetrahydrodeoxycorticosterone (THDOC) (Purdy et al., 1991). Thus, allopregnanolone, 3 $\alpha$ -diol, and THDOC are all produced by the action of the same enzymes (5 $\alpha$ -reductase and 3 $\alpha$ -HSD) with the specific end products determined by whether the substrates are progestogens, androgens, or glucocorticoids. DHP, allopregnanolone, 3 $\alpha$ -diol, and THDOC have very similar biological actions as positive allosteric modulators of GABA<sub>A</sub> receptors (Crawley et al., 1986; Guennoun, 2020; Puia et al., 2003; Reddy & Jian, 2010). GABA<sub>A</sub> receptors are ligand-gated chloride channels whose activation inhibits cells. Accordingly, these neurosteroids have been shown to inhibit cells with GABA<sub>A</sub> receptors (Wang, 2011). Allopregnanolone has been most extensively studied and shown to bind to GABA<sub>A</sub> receptors with delta ( $\delta$ ) subunits, which are the most common extrasynaptic receptors, whose activation increases tonic inhibition (Belelli et al., 2002; Stell et al., 2003).

Like steroid synthesis within neurons and glia, the synthesis of neurosteroids occurs in the mitochondria (Miller & Bose, 2011). Studies have shown that many cells in the brain are equipped with the enzymes necessary to convert

cholesterol or peripheral steroids to neurosteroids (Kimoto et al., 2001), including neurons and glia of the neocortex, hippocampus, amygdala, thalamus and olfactory regions (Agís-Balboa et al., 2006).

### Sex differences in brain synthesis and levels of steroids and neurosteroids

There are obvious sex differences in the peripheral levels of sex steroids and glucocorticoids in both rodents and primates. Female rodents have higher peripheral levels of progestogens, estrogens, and glucocorticoids than males while males have higher peripheral levels of androgens (Nelson & Bulun, 2001; Tyagi et al., 2017). Despite these clear differences and the ease with which these molecules can cross the blood brain barrier, brain concentrations often do not reflect patterns in the periphery in part because of the presence of binding globulins in the blood. A good example of this is the female > male sex difference in peripheral glucocorticoids in rodents, which does not appear to exist in humans (Bangasser & Wicks, 2017; Moisan, 2021; Reschke-Hernández et al., 2017). The exact reason for the species difference in whether males and females exhibit different levels of peripheral glucocorticoids remains unclear, although it is possible that there are sex differences in the regulation of adrenal steroids in rodents that do not exist in humans. Nonetheless, the peripheral sex difference in rodents does not correspond to a similar sex difference in brain glucocorticoid concentrations because females have more corticosteroid binding globulin than males, which limits glucocorticoid access to the brain (Mataradze et al., 1992). Thus, the functional consequences of this rodent sex difference remain unclear.

Another reason that brain concentrations do not reflect peripheral sex differences is brain region-specific expression of enzymes that convert peripherally synthesized steroids into other molecules. Perhaps the best example of this is the sex difference in peripheral estradiol levels in rodents, which is reversed in the brain. Adult female rats and mice have higher levels of circulating estradiol than males, but because of the presence of aromatase in the brain as well as high concentrations of the main substrate of aromatase, testosterone, males have higher concentrations of estradiol in some brain regions (Amateau et al., 2004; Gillies & McArthur, 2010; Hokenson et al., 2022). Taken together, these findings emphasize the need to examine brain concentrations of bioactive molecules directly, rather than inferring their relative concentrations by extrapolating from peripheral levels.

With regard to brain concentrations of sex steroids and glucocorticoids, studies have shown that baseline progestogen levels are higher in female than male rodent brains (Meffre et al., 2007; Pesaresi et al., 2010; Sze et al., 2018). Conversely, baseline androgen concentrations, like estradiol levels, are higher in male than female rodent brains (Hojo & Kawato, 2018; Pesaresi et al., 2010; Sze et al., 2018). By contrast, no baseline differences in brain glucocorticoid concentrations exist between the sexes. The relative concentrations of neurosteroids more closely reflect the concentrations of their precursors, in that female > male sex differences in progesterone

parallel those of allopregnanolone (Meffre et al., 2007), while male > female sex differences in testosterone parallel those in 3 $\alpha$ -diol. In female rodents, the estrous cycle produces fluctuating changes in peripheral progesterone. These estrous cycle differences in progesterone parallel differences in allopregnanolone concentrations with higher levels of during diestrus (Caruso et al., 2010; Laham et al., 2022). Collectively, these findings suggest that brain concentrations of neurosteroids are dependent on both steroid concentrations in the periphery and the presence of enzymes necessary for the production of neurosteroid precursors or neurosteroids themselves.

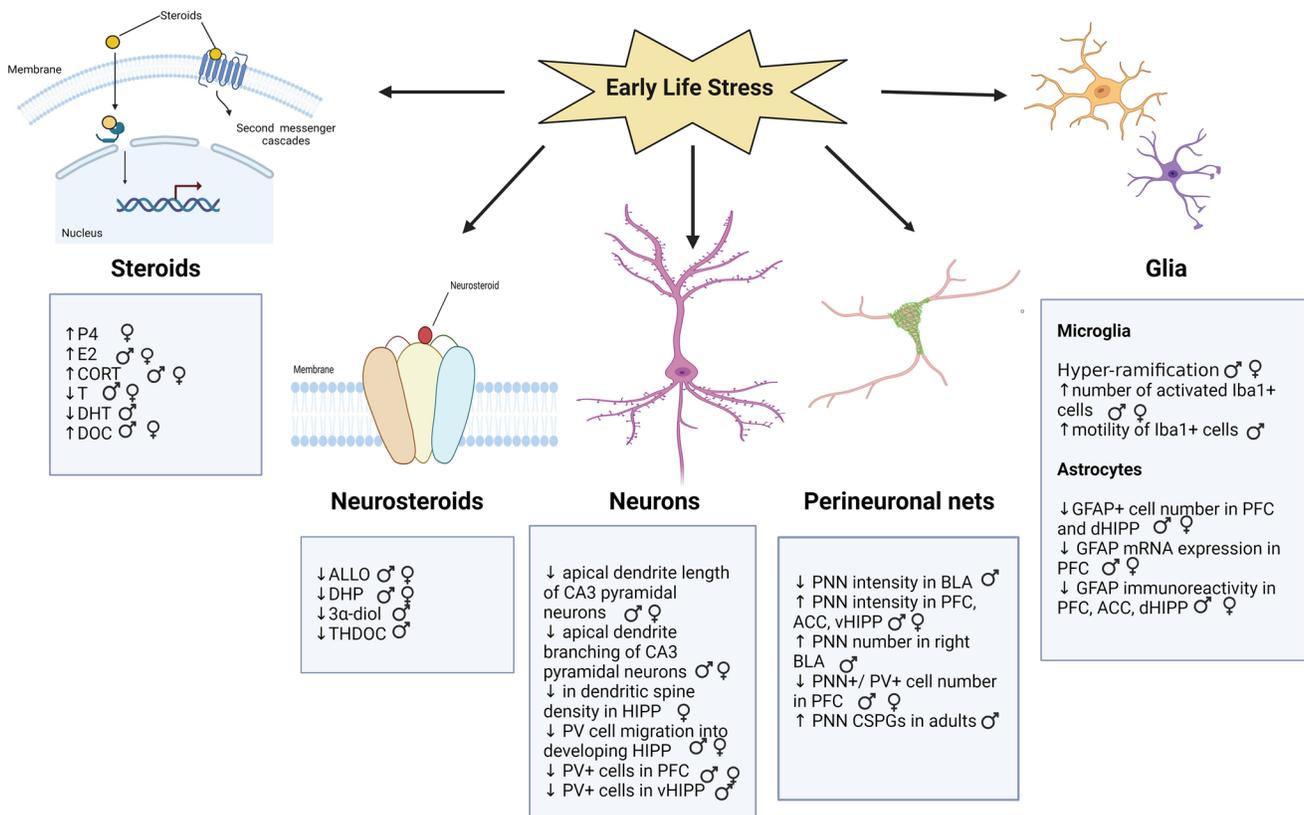
### Impact of stress on sex steroid, glucocorticoid, and neurosteroid levels

Steroid and neurosteroid levels across brain regions fluctuate dynamically in response to different conditions such as stress (Purdy et al., 1991; Barbaccia, 2004). In addition to the baseline differences in neuroactive steroids as described above, sex-specific differences in steroid and neurosteroid levels have been observed in response to stress.

Exposure to adverse conditions during early life alters levels of sex steroids, glucocorticoids, and neurosteroids in rodents and humans (Figure 3). For example, male rats reared under limited bedding and nesting conditions show increased serum estradiol levels (Eck et al., 2022; 2019). Maternal

separation leads to decreased testosterone levels in serum of male mice (Miyaso et al., 2021; Tsuda et al., 2011).

Maternal separation with early weaning has been shown to increase serum and brain concentrations of progesterone in diestrus female mice (Laham et al., 2022). Along with these increases in progesterone levels, allopregnanolone levels are lower (Laham et al., 2022). Changes in the expression of enzymes associated with steroid and neurosteroid synthesis have been reported in response to both prenatal and early postnatal stress in rodents. Juvenile offspring of rat dams exposed to restraint during late gestation showed decreased conversion of progesterone to 5 $\alpha$ -reduced metabolites such as DHP and allopregnanolone in the medial prefrontal cortex (mPFC) (Paris & Frye, 2011b). A reduction in allopregnanolone was noted in the hippocampus of female offspring of rat dams exposed to immune challenge during the late gestation period (Paris et al., 2011). Frye et al. (2010) reported that male rat offspring of dams exposed to prenatal stress show increased corticosterone levels, decreased 3 $\alpha$ -diol concentration, and decreased hippocampal DHT levels accompanied by increased avoidance behavior. Similarly, gestational and acute restraint stress decreased hippocampal 3 $\alpha$ -diol levels and increased avoidance behavior (Walf & Frye, 2012). Our laboratory has found that female diestrus mice subjected to maternal separation and early weaning have reduced levels of 5 $\alpha$ -reductase in the ventral hippocampus, which likely contributes to the elevated ratio of progesterone to



**Figure 3.** Diagram Illustrating Some of the Effects of Developmental Stress on Steroid and Neurosteroid Levels, Neuronal Morphology, Glial Expression, and PNN Integrity. Note: Stress influences various aspects of brain function that extend beyond the factors mentioned in the diagram. P4, Progesterone; E2, Estradiol; T, Testosterone; ALLO, Allopregnanolone; CORT, Corticosterone; DHP, Dihydroprogesterone; DHT, Dihydrotestosterone; DOC, Deoxycorticosterone; THDOC, Tetrahydrodeoxycorticosterone; BLA, Basolateral amygdala; PFC, prefrontal cortex; HIP, hippocampus; dHIP, dorsal hippocampus; vHIP, ventral hippocampus; Iba1, ionized calcium binding adaptor molecule 1; GFAP, Glial fibrillary acidic protein; PNN, Perineuronal nets; PV, Parvalbumin; CSPG, Chondroitin sulfate proteoglycan. ♂: male ♀: female.

allopregnanolone (Laham et al., 2022). Similarly, male juvenile mice reared in isolation have lower expression of 5 $\alpha$ -reductase in the mPFC and nucleus accumbens and reduced allopregnanolone and THDOC in the frontal cortex (Bortolato et al., 2011). Postweaning social isolation has also been shown to result in a downregulation of cortical and plasma concentrations of allopregnanolone, This was also associated with increased avoidance behavior (Serra et al., 2005; Serra et al., 2000). These studies indicate a dysregulation in neurosteroid synthesis in rodent models of developmental stress (Table 2). Although it is difficult to draw connections between studies done in rodents and the human condition, it seems relevant to note that childhood Holocaust survivors have diminished serum levels of 5 $\alpha$ - reductase (Yehuda et al., 2009).

In adulthood, acute stress increases the levels of certain steroids in rodents, including progesterone, testosterone, estrogen, and corticosterone/cortisol, in both the periphery and brain (López-Calderón et al., 1990; Romeo et al., 2007; Shors et al., 1999). Stress increases levels of these steroids, as well as their neurosteroid derivatives, in males and females, with increases in allopregnanolone being greater in the frontal cortex, amygdala and brainstem in females. A significant increase in allopregnanolone was only noted in the frontal cortex in stress-induced male rats (Sze et al., 2018). Studies indicate that plasma and brain levels of THDOC and allopregnanolone rapidly increase in rats exposed to acute stress (Reddy & Rogawski, 2002). Vallée et al. (2000) quantified the neurosteroid concentration in rat plasma and brain following forced swim stress and reported a significant elevation in allopregnanolone and pregnenolone levels. Peak brain and plasma levels of allopregnanolone and THDOC are noted in rats exposed to acute stressors such as cold swim stress, foot shock, or carbon dioxide exposure (Barbaccia et al., 1996, 1997). Similarly, other studies have shown a 4-20 fold rapid elevation in THDOC and allopregnanolone in the rodent cortex and hypothalamus following exposure to acute swim test (Purdy et al., 1991). Investigators have reported a reduction in avoidance behavior in response to the administration of allopregnanolone and THDOC in rodents (Bitran et al., 1991; Wieland et al., 1991). Studies suggest that this increase in the levels of allopregnanolone is a potential homeostatic mechanism, to increase the GABA<sub>A</sub> receptor threshold and promote stress resistance in response to acute challenges (Shirayama et al., 2011; Yoshizawa et al., 2017). Blockade of allopregnanolone production in response to acute stress using finasteride, a 5 $\alpha$ -reductase inhibitor, decreases sensorimotor gating index in response to acute stress, an effect observed in several neuropsychiatric disorders (Pallarès et al., 2015).

Also in adulthood, chronic stress alters levels of peripheral sex steroids and glucocorticoids. Studies have shown that chronic stress elevates serum levels of progesterone and glucocorticoids in male and female rodents, while decreasing levels of testosterone and estradiol in rats and mice (Gao et al., 2020; Retana-Márquez et al., 2003). Studies have also shown that chronic stress-induced changes in peripheral steroid levels do not always parallel changes in neurosteroid concentrations. Indeed, adult male mice exposed to a social isolation paradigm, show a reduction in 5 $\alpha$ -reductase activity, which produces decreased allopregnanolone levels in the

**Table 2.** Impact of Stress on Sex Steroid, Glucocorticoid, and Neurosteroid Levels.

| Time of Stress | Stressor                          | Species/Sex                                    | Brain Region                                 | Sex Steroid/Glucocorticoid/Neurosteroid   | Change in Levels  | Associated Behavioral Changes   | Reference   |
|----------------|-----------------------------------|--|--|---|---|---|---|
| Early Life     | LBN                               | Rats/Male and Female                           | N/A (serum levels)                           | E2  | Increased in males  | Shorter latency to engage in reproductive behavior  | Eck et al., 2020, 2022  |
|                | MS                                | Mice/Male                                      | N/A (serum levels)                           | T   | Decreased   | Decreased aggressive behavior   | Miyaso et al., 2021; Tsuda et al., 2011   |
|                | MSEW<br>Restraint- Late Gestation | Mice/Female (Diestrus)<br>Rats/Male and Female | vHIP<br>mPFC, HIP                            | P4, ALLO<br>P4 to 5 $\alpha$ -reduced metabolites (DHP, ALLO),<br>3 $\alpha$ -diol, DHT | Increased P4, Decreased ALLO<br>Decreased Conversion to 5 $\alpha$ -reduced metabolites. Decreased 3 $\alpha$ -diol, DHT in males | Increased avoidance<br>Impaired object recognition, Increased avoidance, reduced social interaction | Laham et al., 2022<br>Paris & Frye, 2011b; Waif & Frye, 2012  |
| Adulthood      | LPS – Late Gestation              | Rats/Female                                    | HIP, PFC                                     | ALLO  | Decreased   | Impaired object recognition   | Paris et al., 2011  |
|                | SI                                | Mice/Male, Rats/Male                           | mPFC, nACC, HIP, OB                          | ALLO, THDOC, 5 $\alpha$ - reductase<br>P5, P4, ALLO, THDOC, DHP                         | Decreased   | Increased avoidance and aggression  | Bortolato et al., 2011; Serra et al., 2005, 2000; Dong et al., 2001;<br>Pinna et al., 2004  |
|                | Swim, CO <sub>2</sub>             | Rats/Male and Female                           | FC, AMY, Brainstem, HYPO, serum              | ALLO, DOC, P4, THDOC  | Increased (More in Females)<br>Decreased ALLO in serum  | Disrupted prepulse inhibition   | Sze et al., 2018; Reddy & Rogawski, 2002; Vallée et al., 2000; Barbaccia et al., 1996; Purdy et al., 1991;<br>Pallarès et al., 2015 |
|                | CUS<br>Restraint; Shock, Swim     | Mice/Female<br>Rats/Male                       | N/A (serum measures)<br>N/A (serum measures) | T, E2<br>T  | Decreased<br>Decreased  | Not reported<br>Greater latency to engage in reproductive behavior                                  | Gao et al., 2020<br>Retana-Márquez et al., 2003;  |

\*Compared to unstressed controls.

LBN, limited bedding and nesting; MS, maternal separation, MSEW, maternal separation and early weaning; SI, social isolation; DHP, dihydroprogesterone; DOC, deoxycorticosterone; DHT, dihydrotestosterone; DHT, dihydrotestosterone; DOC, deoxycorticosterone; THDOC, tetrahydrodeoxycorticosterone; T, testosterone; E2, estradiol; ALLO, allopregnanolone; P4, progesterone; P5, pregnenolone; PFC, prefrontal cortex; HIP, hippocampus; ACC, anterior cingulate cortex; OB, olfactory bulb; FC, frontal cortex; AMY, amygdala; HYPO, hypothalamus.

frontal cortex and olfactory bulb. These findings were not observed in female mice (Dong et al., 2001; Pinna et al., 2004). The available evidence suggests that compensatory mechanisms exist in the brain to mitigate peripheral changes in steroid levels after acute stress. These mechanisms appear to be diminished after chronic stress, possibly through a downregulation of enzymes that convert steroids to neurosteroids with buffering action on avoidance behavior (Table 2). In humans, individuals with neuropsychiatric disease associated with stress, including posttraumatic stress disorder and major depressive disorder, have lower levels of serum 5 $\alpha$ -reductase (Agis-Balboa et al., 2014), which likely influences levels of neurosteroids, as some studies have suggested (Römer et al., 2010; Römer & Gass, 2010).

### Impact of stress on neurons: involvement of steroids and neurosteroids

Stress is known to have profound effects on neuronal excitability, biochemistry, and structure, some of which are mediated by stress-induced changes in steroids and neurosteroids. The most obvious steroids to be implicated in stress effects on neurons are glucocorticoids, also known as stress hormones. A large literature has shown that developmental stress influences neurotransmission, gene expression, dendritic architecture, and postnatal neurogenesis (De Kloet et al., 1996; McEwen et al., 2016; Mirescu et al., 2004; Monroy et al., 2010). For example, prenatal and postnatal stress, as well as chronic stress in adulthood, result in atrophied apical dendrites of CA3 pyramidal cells and diminished hippocampal, but not prefrontal or hypothalamic, synaptic markers in rodents (Liu et al., 2016; Paris & Frye, 2011a; Watanabe et al., 1992). Some of these effects have been attributed to changes in glucocorticoids (De Kloet et al., 1996; McEwen et al., 2016; Mirescu et al., 2004) and others are very similar to what has been observed with chronic stress in adulthood, where a causal link with glucocorticoids has been established (Horchar & Wohleb, 2019; Kvarta et al., 2015) (Table 3). However, it should be emphasized that elevated glucocorticoid levels do not explain many of the persistent effects of early life adversity as well as those of stress in adulthood (Faturi et al., 2010; Kim et al., 2015).

In addition to glucocorticoids, other steroids known to be altered by developmental stress, as well as acute and chronic adult stress, influence neurons and as such, may contribute to stress responses. For example, estrogen and progesterone, which are both elevated in response to postnatal and acute adult stress in rodents (Paris & Frye, 2011a, 2011b; Shors et al., 1999), are known to increase dendritic spine density in the hippocampus (Gould et al., 1990; Woolley et al., 1990). Allopregnanolone has a similar effect to progesterone of increasing dendritic spine density (Barreto-Cordero et al., 2020; Shimizu et al., 2015) raising the possibility that progesterone acts through its derivative allopregnanolone to modulate dendritic spines. Studies have shown that ovariectomized female rats treated with estrogen replacement and subjected to acute stress showed an increase in spine density in basolateral amygdala (BLA) (Shansky et al., 2010). A similar increase in BLA spine density was noted in male rats exposed to chronic stress (Mitra et al., 2005). In adulthood, acute stress exerts

**Table 3.** Impact of Stress on Neurons: Involvement of Steroids and Neurosteroids.

| Time of Stress | Stressor                            | Species/Sex                                | Brain Region     | Sex Steroid/Glucocorticoid/Neurosteroid | Affected Neuron Type or Area   | Effect on Neurons   | Reference  |
|----------------|-------------------------------------|--|------------------|---|--|---|--|
| Early Life     | MS, MS+SI                           | Rats/Male and Female; Mice/Male and Female | HIP, PFC         | CORT                                    | Immature neurons of dentate gyrus; PV+ interneurons                  | Reduced number of new neurons and PV+ neurons, as well as suppressed maturation of PV+ neurons                              | Mirescu et al., 2004; Katahira et al., 2018  |
|                | Multiple stressors                  | Rats/Male and Female                       | dHIP, mPFC, HYPO | DHP, P4, E2                             | Pyramidal cells  | Decrease in spine density in dHIP, no differences in spine density noted in mPFC and HYPO                                   | Paris & Frye, 2011a  |
| Adulthood      | Shock                               | Rats/Male and Female                       | HIP              | E2                                      | CA1 pyramidal cells  | Increased Dendritic Spine Density   | Shors et al., 1999; Shors et al., 2001   |
|                | Restraint, multiple acute stressors | Rats/Male and Female                       | HIP              | E2 (in females), Glucocorticoids        | Pyramidal cells  | Decreased dendritic spine density, apical dendritic length and complexity   | Hokenson et al., 2021; Watanabe et al., 1992; Luine et al., 1994                     |
|                | Restraint, SI                       | Rats/Male and Female                       | HIP, AMY         | CORT, E2                                | PV+ interneurons of CA1, CA3, and dentate gyrus; BLA pyramidal cells | Chronic stress induced decrease in number; Increase in spine density; Increase in dendritic length at intermediate branches | Filipović et al., 2013; Kraus et al., 2022; Mitra et al., 2005; Shansky et al., 2010 |

\*Compared to unstressed controls.

MS, maternal separation; SI, social isolation; CORT, corticosterone; E2, estradiol; P4, progesterone; DHP, dihydroprogesterone; PV, parvalbumin; BLA, basolateral amygdala; HIP, hippocampus; PFC, prefrontal cortex; AMY, amygdala; HYPO, hypothalamus.

differential effects on CA1 pyramidal cell dendritic spine density in male and female rats (Shors et al., 1999). Stress reduces dendritic spine density in female rats when they are in proestrus, while it has the opposite effect in male rats (Shors et al., 2001). Similar results were observed in female mice where acute stress in the context of higher estrogen leads to reduced dendritic spine density and impaired hippocampus-dependent memory, while similar stress exposure during a low estrogen state has no effects (Hokenson et al., 2021). Furthermore, while acute stress in adulthood increases dendritic spines in males, developmental stress has the opposite effect in male mice and rats (Monroy et al., 2010; Xu et al., 2022) (Table 3). These findings suggest a complex interplay between stress effects and sex that may be not only dependent on baseline levels of steroids and neurosteroids but also on the age at which the stress occurs.

Testosterone also influences dendritic spine density in the hippocampus with parallel results in performance on hippocampus-dependent learning tasks in rats (Muthu et al., 2022). Studies have also shown that both testosterone and its derivative DHT can stimulate hippocampal spine density in rats (Hatanaka et al., 2015). Taken together, these findings raise the possibility that stress-induced changes in progestogens, androgens, estrogens, and glucocorticoids, as well as their neurosteroid derivatives may be responsible for some stress-induced effects on neurons, including on dendritic structure.

In addition to changes in excitatory neurons, developmental and adult stress have been shown to influence inhibitory interneurons in both the hippocampus and prefrontal cortex. Maternal separation diminishes the migration of parvalbumin positive (PV+) interneurons into the developing hippocampus (Katahira et al., 2018). Similarly, exposure to early life adversity has been shown to reduce the number of PV+interneurons in the prefrontal cortex of female rats (Gildawie et al., 2021). We have found a reduction in the number of PV+interneurons, and somatostatin+interneurons, in the ventral hippocampus of adult male mice previously subjected to maternal separation with early weaning (Murthy et al., 2019) although in the case of PV+cells, this reduction likely reflects diminished production of parvalbumin rather than an actual decrease in the number of these cells. Studies in adult rats have shown that chronic, but not acute, stress also reduces parvalbumin expression in hippocampus interneurons (Filipović et al., 2013) (Table 3). Although inhibitory interneurons, including PV+cells, are known to express GRs and ERs (Hernández-Vivanco et al., 2022; Kraus et al., 2022), it remains unexplored whether the effects of stress on this population are mediated through these signaling mechanisms.

Likewise, although both principal neurons and inhibitory interneurons express GABA<sub>A</sub> receptors, which are known targets of neurosteroid derivatives of sex steroids and glucocorticoids, the connection between stress effects and neurosteroid action remains unexplored.

### Impact of stress on perineuronal nets: involvement of steroids and neurosteroids?

Many neurons shown to be affected by early life stress are enwrapped in perineuronal nets (PNNs), extracellular matrix

structures known to regulate neural underpinnings of cognition and behavior, including but not limited to, synaptic plasticity, neuronal oscillations, and synchrony across brain regions (Sorg et al., 2016; Wingert & Sorg, 2021). Numerous studies have investigated the impact of postnatal and adult stress on PNNs and shown complex effects that seem to depend on multiple factors, including brain region, sex, and timing/type of stressor (Laham & Gould, 2021).

Postnatal stress using the maternal separation and early weaning stress paradigm has been shown to increase PNN intensity in the ventral hippocampus in adulthood along with increases in avoidance behavior (Catale et al., 2022; Dimatelis et al., 2013; Murthy et al., 2019). An upregulation of PNN CSPGs such as tenascin, brevican, neurocan was also noted in animals subjected to maternal separation (Dimatelis et al., 2013). Other results suggest an increase in PNN number in the BLA and HIP animals exposed to stress during the postnatal period or in adulthood (Guadagno et al., 2020; Riga et al., 2017), including an adult paradigm of enrichment loss (Smail et al., 2023). However, additional studies have shown a downregulation in PNN intensity in PFC, BLA (Gildawie et al., 2021; Santiago et al., 2018) and PNN number (Gildawie et al., 2020; Klimczak et al., 2021; Ueno et al., 2018; Yu et al., 2020) in PFC, HIP, prelimbic cortex, and BLA in animals exposed to postnatal and adult stress paradigms such as maternal separation, social isolation, social defeat, and chronic mild stress. Sex-dependent effects of postnatal stress have also been observed in the development of PNNs around PV+cells in the PFC and BLA of male and female rats, with a higher PNN intensity noted in the PFC of male rats exposed to maternal separation and juvenile social isolation, while a higher PNN intensity reported in BLA of female rats (Gildawie et al., 2021). Similarly, increase in PNN intensity was reported in the right amygdala of male juvenile rats but not females after exposure to early life stress (Guadagno et al., 2020). Studies have shown that changes in PNN intensity/number are associated with increased in avoidance behavior and impairments in stress regulation (Laham et al., 2022; Lee & Lee, 2021; Murthy et al., 2019; Santiago et al., 2018; Yu et al., 2020). Collectively, these studies suggest that a dysregulation in PNN intensity and number occurs after exposure to several stress paradigms. It is important to consider factors such as type of stressor, duration of time after stress exposure (Gildawie et al., 2020, 2021; Laham & Gould, 2021; Ueno et al., 2018), sex, and stage of development (Drzewiecki et al., 2020) when addressing the impact of stress on PNNs (Table 4).

The potential involvement of steroids and neurosteroids in stress-induced PNN changes remains relatively unexplored. Studies have shown that chronic corticosterone treatment in adult mice leads to an increase in PNN intensity in the hippocampus (Alaiyed et al., 2020), which is consistent with the possibility that stress-induced increases in glucocorticoids, or their derivatives, influence PNNs. Studies have shown that inhibition of estradiol synthesis increases PNN intensity around PV+ interneurons in the dorsal CA1 region of the hippocampus in female mice, a finding that has been causally linked to diminished hippocampus-dependent learning and memory and hippocampal neuronal oscillations (Hernández-Vivanco et al., 2022). Given that studies have

**Table 4.** Impact of Stress on Perineuronal Nets: Involvement of Steroids and Neurosteroids?.

| Time of Stressor | Stressor      | Species/Sex                                   | Brain Region        | PNN Change   | Associated Behavioral Changes  | Reference   |
|------------------|---------------|---|---------------------|--|--|---|
| Early Life       | MSEW          | Mice/Male and Female;<br>Rats/Male and Female | HIP, BLA, PFC       | Increased PNN Intensity; Increased PNN CSPGs; Affects PNN Composition (increased C4S+/WFA+ cells in ventral CA1) | Increased avoidance  | Murthy et al., 2019; Dimatelis et al., 2013; Laham et al., 2022 |
|                  | MS, SI        | Rats/Male and Female                          | PFC                 | Reduced PV + PNN structural integrity, PV + PNN reduced number in females  | risk assessment in females   | Gildawie et al., 2021   |
|                  | LBN<br>LBN    | Rats/Male and Female<br>Rats/Male             | BLA<br>BLA          | Increased PNN density around PV+ cells<br>Reduced PNN intensity  | Increased locomotion, increased risk assessment in females<br>Increased fear | Guadagno et al., 2020<br>Santiago et al., 2018                  |
| Adulthood        | Social Defeat | Rats/Male and Female                          | HIP                 | Increased PNN number around PV+ cells  | Reduced object location memory and maintenance of LTP                        | Riga et al., 2017   |
|                  | SI<br>CMS     | Mice/Male<br>Rats/Male                        | HIP, RC; PFC<br>PFC | Reduced PNN number; Reduction in PNN number in PFC<br>Reduced PNN number   | N/A  | Klimczak et al., 2021; Ueno et al., 2018                        |
|                  | Chronic CORT  | Mice/Male and Female                          | HIP                 | Increased PNN intensity  | Depressive-like behavior<br>Increased avoidance; reduced working memory      | Yu et al., 2020<br>Alayed et al., 2020                          |

\*Compared to unstressed controls.

MSEW: Maternal separation and early weaning; MS, maternal separation; SI, social isolation; LBN: limited bedding and nesting; CMS, chronic mild stress; PNN, perineuronal nets; CSPG, chondroitin sulfate proteoglycan; C4S, chondroitin-4-sulfate; WFA, wisteria floribunda agglutinin; LTP, long-term potentiation; PFC, prefrontal cortex; HIP, hippocampus; BLA, basolateral amygdala; RC, retrosplenial cortex.

shown early life and adult stress affect estradiol levels, it is possible that some stress effects may exert their action through modulation of circulating or brain-derived estradiol although the possibility remains to be investigated. Allopregnanolone inhibition in adult female mice has been shown to alter PNN composition in the ventral CA1, increasing the numbers of PNNs that contain the C4S sulfation pattern, a PNN characteristic known to inhibit plasticity (Laham et al., 2022). Similar changes were observed in ventral CA1 PNNs in adult female mice after early life stress (maternal separation with early weaning), an experience that diminishes ventral hippocampal allopregnanolone concentrations (Laham et al., 2022). Taken together, these findings suggest that early life stress may alter PNN composition through actions on allopregnanolone. This is an area that is clearly in need of further investigation. It is also worth noting that neuronal activity has been shown to modulate PNN intensity (Evans et al., 2022), thus, the influence of these molecules on PNNs may occur indirectly, through effects on neuronal function.

### Impact of stress on glia: involvement of steroids and neurosteroids?

Microglia and astrocytes are highly sensitive to environmental changes and involved in inflammatory processes. Several studies indicate that stress may be associated with the dysregulation of immune system response (Black, 2003; Kiecolt-Glaser et al., 1996). Microglia respond to stress in a variety of ways, including increasing in number, producing pro-inflammatory cytokines, and expressing cell surface antigens (Calcia et al., 2016; Kettenmann et al., 2011). One of the key cell surface antigens is the intracellular ionized calcium-binding adaptor protein, Iba1, which is found in microglia and is upregulated during inflammation. Upregulation in Iba1 expression in the hippocampus has been reported in rodents exposed to different stress paradigms when compared to unstressed animals (Bian et al., 2012; Kojo et al., 2010; Park et al., 2011; Wohleb et al., 2011). A similar increase in Iba1 expression is observed in other regions of the brain such as the amygdala, nucleus accumbens, and PFC in animals subjected to stress (Bian et al., 2012; Schiavone et al., 2009; Tynan et al., 2010; Wohleb et al., 2011) (Table 5).

Maternally separated male and female rats exposed to a "second hit" of stress during adulthood, show an upregulation of Iba1 activity in the PFC, along with hyper-ramified microglial morphology, indicating microglial activation or a priming process increasing the susceptibility of a heightened response during subsequent stressors (Ganguly et al., 2018; Hoeijmakers et al., 2017; Roque et al., 2016; Saavedra et al., 2017; Tay et al., 2018). Takatsuru and colleagues report that ELA increases the number and motility of microglial processes (Takatsuru et al., 2015). Additionally, exposure to early life adversity results in a decrease in the arborization area of Iba1+ cells (Baldy et al., 2018). During normal hippocampus growth in mice, there is a decrease in microglial density and phagocytic activity. However, in mice exposed to ELA, there is an increase in phagocytic activity during hippocampal development as well as an increase in microglial density and surface area in the hippocampus at PND 14 (Delpech et al., 2016) (Table 5).

**Table 5.** Impact of Stress on Microglia and Astrocytes: Involvement of Steroids and Neurosteroids?

| Time of Stressor | Stressor                                    | Species/Sex   | Brain Region   | Changes in Microglia/Astrocytes   | Reference   |
|------------------|---|---|--|---|---|
| Early Life       | MS  | Rats/Male and Female;<br>Mice/Male and Female                                     | HIP; SSCx;<br>Medulla; PFC; nACC,<br>dorsal striatum             | Increased activated microglia, decreased astrocyte density and number of processes; Increased motility of microglial processes in SSCx; Increased size of microglial soma and decreased arborization in medulla; Decreased astrocytes | Roque et al., 2016; Takatsuru et al., 2015; Baldy et al., 2018; Delpech et al., 2016; Giridharan et al., 2019, Réus et al., 2017, 2019; Banqueri et al., 2019 |
|                  | MS and FR<br>MS and LPS<br>MS and SI<br>LBN | Rats/Male and Female<br>Rats/Male<br>Rats/Male and Female<br>Mice/Male and Female | PFC<br>HIP<br>PFC<br>HIP, entCx                                  | Increased Iba1, hyper-ramified microglia<br>Increased microglial activation, decreased astrocytes<br>Decreased GFAP mRNA<br>Increased astrocyte coverage at P9, and a decrease noted at 10 months                                     | Ganguly et al., 2018<br>Saavedra et al., 2017<br>Yamawaki et al., 2018<br>Abbink et al., 2020   |
| Adulthood        | CUS   | Mice/Male; Rat/Male and Female  | HIP; PFC   | Increased activated astrocytes in HIP; atrophy of astrocyte processes; Decreased GFAP mRNA levels in PFC; decreased astrocytes in HIP   | Bian et al., 2012; Li et al., 2013; Liu et al., 2009, 2011; Lou et al., 2018; Tynan et al., 2013  |
|                  | Restraint                                   | Gerbil/ Male; Rat/Male and Female   | HIP  | Hypertrophied and activated microglia; Increase in microglia density, number  | Park et al., 2011; Tynan et al., 2010   |
|                  | Restraint and Swim                          | Mice/Male and Female  | Thalamus, HYPO, HIP,<br>Substantia nigra,<br>central gray<br>HIP | Increased microglial activation   | Sugama et al., 2007   |
|                  | Shock                                       | Rats/Male and Female  | HIP  | Increased microglia, no change in astrocytes; reduced microglial phagocytic activity  | Frank et al., 2007; Fonken et al., 2018   |
|                  | Social stress<br>Psychosocial stress<br>SI  | Mice/Male<br>Tree shrew/Male<br>Rats/Male   | AMY, HIP, PFC<br>HIP<br>nACC, PFC                                | Increased de-ramified microglia<br>Decreased astrocytes and soma volume<br>Increased microglia  | Wohleb et al., 2011<br>Czéh et al., 2006<br>Schiaivone et al., 2009   |

\*Compared to unstressed controls.

MS, maternal separation; MSEW, maternal separation and early weaning; FR, food restriction; LPS, lipopolysaccharide; SI, social isolation; LBN, limited bedding and nesting; CUS, chronic unpredictable stress; Iba1, ionized calcium binding adaptor molecule-1; GFAP, Glial fibrillary acidic protein; PFC, prefrontal cortex; HIP, hippocampus; SSCx, somatosensory cortex; nACC, nucleus accumbens; entCx, entorhinal cortex; HYPO, hypothalamus; AMY, amygdala.

In adulthood, acute stress increases microglial number (Frank et al., 2007), and induces de-ramification (Sugama et al., 2007) and according to more recent studies, adult exposure to acute stress reduces microglial phagocytic activity. (Fonken et al., 2018; Frank et al., 2019). Chronic stress in adulthood causes inflammation, which is characterized by an increase in brain cytokine levels, and this elevation is associated with changes in microglia morphology (Calcia et al., 2016; Yirmiya et al., 2015).

Exposure to chronic stress is associated with an increase in Iba1+ cell density, Iba1 area and microglial phagocytic function (Frank et al., 2019; Hinwood et al., 2012; Nair & Bonneau, 2006; Tynan et al., 2010). Microglia hyper-ramification has also been observed in the PFC and hippocampus of animals subjected to chronic restraint stress (Hinwood et al., 2013) or repeated swimming tests (Hellwig et al., 2016), while an increase in de-ramified microglia were noted in the amygdala, PFC and hippocampus of mice subjected to repeated social defeat paradigm (Wohleb et al., 2011) (Table 5).

Stress hormones and cytokines influence glial fibrillary acidic protein (GFAP), a cytoskeletal astroglia protein, and an increase in these levels indicates astrocyte activity. An upregulation in astrocytic activity and neuroinflammatory cytokines was reported in animals subjected to early life stress paradigm such as maternal deprivation (Giridharan et al., 2019; Réus et al., 2017, 2019). Conversely, a decrease in GFAP mRNA expression and GFAP+ cell number was noted in the PFC of mice exposed to maternal separation (Musholt et al., 2009; Yamawaki et al., 2018).

Additionally, a decrease in GFAP immunoreactivity was reported in the PFC, ACC, dorsal hippocampus of animals exposed to ELA (Abbink et al., 2020; Banqueri et al., 2019). In adulthood, exposure to acute stressors resulted in a decrease in astrocyte density (Saur et al., 2016), GFAP expression in the hippocampus (Xia et al., 2013) and induces astrocytic hypertrophy (Murphy-Royal et al., 2019). Chronic stress in adulthood is associated with a reduction in astrocyte number and somatic volume in the hippocampus (Altshuler et al., 2010; Banasr et al., 2010; Czéh et al., 2006; Li et al., 2013, Liu et al., 2009, Liu et al., 2011, Tynan et al., 2013). Exposure to chronic mild unpredictable stress during adulthood is also associated with a decrease in structural complexity of astrocytes (Yamawaki et al., 2018) (Table 5).

The involvement of steroids and neurosteroids in stress-induced changes in microglia and astrocytes has been incompletely explored, but chronic stress-induced changes in microglial activation and astrocyte number can be blocked by GR antagonism (Horchar & Wohleb, 2019; Lou et al., 2018) and stress-related memory formation can be blocked by deleting GRs from astrocytes (Tertel et al., 2018; Wiktorowska et al., 2021). Evidence showing that allopregnanolone increases microglial cell processes and decreases microglia migration in mice, suggests a role for allopregnanolone in modulating microglial morphology and inhibiting phagocytic function. (Jolivel et al., 2021). Given these insights into the interplay between glial cells and neurosteroids, and considering the known effects of stress on these entities individually, it seems increasingly important to explore the impact of stress on glial functionality through the modulation of steroids and neurosteroids.

## Conclusions

Early adverse experiences are known to alter neurons, glia, and the extracellular matrix and many of these effects have been causally linked to behavioral change that lasts into adulthood. Understanding the connection between aversive experience and brain changes will require considering all these brain components, as well as the many signaling molecules that can be altered by early life stress. Early adversity is known to alter the levels of glucocorticoids and sex steroids in both the periphery and brain, and these molecules can be synthesized in both compartments. While studies have investigated the causal role of glucocorticoids in stress effects, less attention has been paid to the potential involvement of glucocorticoid derivatives, as well as to sex steroids produced in the periphery and brain, and their neurosteroid end products. Additional studies directly investigating the potential involvement of these signaling molecules in stress influences on neurons, glia, and the extracellular matrix will help to shed light on the complex cascade of events that produce lasting changes after early life stress.

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The authors report there are no competing interests to declare.

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