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The role of ovarian hormone-derived neurosteroids on the regulation of GABA_A receptors in affective disorders

Georgina MacKenzie¹ and Jamie Maguire¹

¹Tufts University School of Medicine, Department of Neuroscience, Boston, MA 02111

Abstract

Rationale—Neuroactive derivatives of steroid hormones, neurosteroids, can act on GABA_A receptors (GABA_ARs) to potentiate the effects of GABA on these receptors. Neurosteroids become elevated to physiologically relevant levels under conditions characterized by increased steroid hormones. There is considerable evidence for plasticity of GABA_ARs associated with altered levels of neurosteroids which may counteract the fluctuations in the levels of these allosteric modulators.

Objectives—The objective of this review is to summarize the current literature on GABA_AR plasticity under conditions characterized by alterations in neurosteroid levels, such as over the estrous cycle, during puberty, and throughout pregnancy and the postpartum period.

Results—The expression of specific GABA_AR subunits are altered over the estrous cycle, at puberty, and throughout pregnancy and the postpartum period. Inability to regulate δ subunit-containing GABA_ARs throughout pregnancy and the postpartum period is associated with depression-like behavior restricted to the postpartum period.

Conclusions—GABA_AR plasticity associated with alterations in neurosteroid levels represents a homeostatic compensatory mechanism to maintain an ideal level of inhibition to offset the potentiating effects of neurosteroids on GABAergic inhibition. Failure to properly regulate GABA_ARs under conditions of altered neurosteroid levels may increase vulnerability to mood disorders, such as premenstrual syndrome (PMS), premenstrual dysphoric disorder (PMDD), and postpartum depression.

Keywords

Neurosteroids; GABA receptors; ovarian hormones; allopregnanolone; estrous cycle; pregnancy; postpartum

Introduction

The ovarian derived sex hormones, estrogen, progesterone and testosterone mediate a number of important physiological functions including reproductive/sexual behaviors and the maintenance of pregnancy (Cunningham et al. 2012; Graham and Clarke 1997;

Micevych and Sinchak 2008). Peripherally derived steroid hormones are lipophilic and readily cross the blood brain barrier where they can act at the genomic level via steroid receptors to modify neuronal activity and ultimately animal behavior (Cunningham et al. 2012; Graham and Clarke 1997; Handa et al. 2012). Whilst genomic changes are slow to implement (minutes to hours); progesterone and other peripheral steroids can have rapid effects on neuronal excitability following their metabolism in the brain to neurosteroids (Bitran et al. 1993; Mellon and Griffin 2002; Paul and Purdy 1992). The principle target of neurosteroids, such as the progesterone metabolite allopregnanolone, is the GABA_A receptor (GABA_AR) family where they have been shown to have anxiolytic, anti-convulsant, sedative/anesthetic, and analgesic properties ((Belelli et al. 2009; Belelli and Lambert 2005). Alterations in the level of steroid hormones, such that occur over the estrous cycle, at puberty, and during pregnancy and the postpartum period, have been implicated in triggering affective disorders, such as premenstrual dysphoric disorder (PMDD), postpartum depression and mood changes associated with puberty (Rapkin and Akopians 2012; Smith 2013) (Brummelte and Galea 2010). This review will highlight the evidence suggesting that neurosteroid-mediated alterations in GABAergic inhibition are associated with affective disorders, whilst highlighting the areas of therapeutic promise.

I. Neurosteroidogenesis

Neurosteroids are synthesized de novo in the brain from cholesterol (for review see (Do Rego et al. 2009). However, due to their lipophilic nature, progesterone and other peripherally derived steroid hormones readily cross the blood brain barrier where they can be locally metabolized to neurosteroids. Once in the brain, progesterone can be metabolized to allopregnanolone by the consecutive enzymatic actions of 5 α -reductase and 3 α hydroxysteroid dehydrogenase (3 α -HSD). The conversion of progesterone to allopregnanolone in the brain requires the availability of the necessary steroidogenic enzymes which are not universally expressed in all cell types or brain regions. In the mouse, 5 α -reductase and 3 α -HSD mRNA have both been detected in principal neuronal populations in the hippocampus, cortex, amygdala, thalamus, cerebellum and striatum suggesting that these are also neurosteroidogenic sites (Agis-Balboa et al. 2006); whereas, only limited expression was found in interneuron populations (Agis-Balboa et al. 2006). Although it appears that neurosteroids are synthesized in principal neurons, it is unclear whether neurosteroids act on neurons in a paracrine or autocrine fashion (for review see (Lambert et al. 2009)).

In addition to the local metabolism of progesterone in the brain, allopregnanolone is also synthesized in the periphery where, once it enters the brain, it can influence neuronal excitability. In fertile women, allopregnanolone is synthesized in the corpus luteum peaking in the luteal phase with serum levels reaching up to 4 nmol/L compared to < 1 nmol/l in the follicular phase (Andreen et al. 2009; Bicikova et al. 1998; Genazzani et al. 1998; Monteleone et al. 2000; Purdy et al. 1990; Wang et al. 1996). Experimental evidence confirms the expression of the necessary steroidogenic enzymes 5 α -reductase (Haning, Jr. et al. 1996; Ottander et al. 2005) and 3 α -HSD mRNA in human ovarian tissue (Ottander et al. 2005), supporting its role in the cyclic changes in neurosteroid levels. Interestingly, allopregnanolone is still detected in the brain in ovariectomized rats (Paul and Purdy 1992),

further demonstrating that local *de novo* neurosteroid synthesis also occurs in the brain. During pregnancy, however, the majority of allopregnanolone is synthesized in the placenta. Serum allopregnanolone measurements range from 40 to >100nM in both rats (Concas et al. 1998) and humans (Gilbert Evans et al. 2005; Hill et al. 2007; Luisi et al. 2000; Paul and Purdy 1992; Pearson Murphy et al. 2001) during pregnancy, which rapidly drop upon parturition. Levels in the brain may be even higher due to local neurosteroid synthesis, diffusion barriers and differences in metabolism (Mackenzie and Maguire 2013). In addition, the potential for neurosteroids to accumulate in the membrane will not only increase their local concentration but may also prolong their effects due to the slow release of steroids from these internal stores (For review see (Akk et al. 2009)). Although allopregnanolone is largely considered to be an ovarian-derived neurosteroid, with fluctuations occurring over the estrous cycle and during pregnancy, elevations in allopregnanolone have also been observed following stress (Purdy et al. 1991; Purdy et al. 1992). Periods characterized by fluctuations in neurosteroid levels are particularly vulnerable periods for the development of affective disorders, including PMDD and postpartum depression, which may involve the inability to properly regulate GABA_ARs, the main neuronal target of neurosteroids (for review see (Maguire and Mody 2009)).

II. Modulatory effects on GABA_ARs

The GABA_AR family consists of 19 subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ , π and ρ 1–3) which assemble to form a pentameric structure around a chloride and bicarbonate permeable central pore. The specific receptor subunit combination (which usually consists of two α , two β and either a γ or δ subunit (Farrar et al. 1999; Tretter et al. 1997)) determines both the channels biophysical and pharmacological properties along with its subcellular localization. For instance, γ_2 containing receptors are predominately localized at the synapse where they mediate rapid synaptic (“phasic”) inhibition (Farrant and Nusser 2005). However, receptors containing the δ subunit are localized extrasynaptically where they bind GABA with high affinity but low efficacy to generate a persistent “tonic” form of inhibition (Farrant and Nusser 2005). Whereas the γ_2 subunit is widely expressed (Pirker et al. 2000; Wisden et al. 1992), expression of the δ subunit is mainly restricted to the cortex, hypothalamus, cerebellum, dentate gyrus, striatum and thalamus (Hortnagl et al. 2013; Peng et al. 2002; Peng et al. 2004; Pirker et al. 2000; Sarkar et al. 2011; Wisden et al. 1992) where it preferentially pairs with the α_4 subunit (except in the cerebellum where it pairs with α_6) (Olsen and Sieghart 2008). Neurosteroids are potent allosteric modulators of GABA_ARs whilst at sufficiently high concentrations they can directly activate the receptor in the absence of GABA (Majewska et al. 1986). Although neurosteroids are active at all major GABA_AR isoforms, the addition of the δ subunit confers sensitivity to low nanomolar concentrations of neurosteroids in both recombinant expression systems and *in vitro* slice recordings (Houston et al. 2012; Stell et al. 2003). Indeed, neurosteroid sensitivity is greatly reduced in mice which lack the GABA_AR δ subunit (*Gabrd*^{-/-} mice) (Mihalek et al. 1999; Sarkar et al. 2011; Spigelman et al. 2003; Vicini et al. 2002). Interestingly, the neurosteroid binding site is independent of the GABA_AR δ subunit. Rather, the conserved neurosteroid binding sites responsible for both allosteric potentiation and direct receptor activation of GABA_ARs are found on the interface of the α and β subunits (Hosie et al. 2006). As GABA

is a poor agonist at δ subunit-containing receptors, the greater potency of neurosteroids at these receptors is not due to the δ subunit itself but rather to the ability of neurosteroids to increase the efficacy of GABA at these receptors which is less apparent in isoforms where GABA is already an effective agonist (Bianchi and Macdonald 2003). At higher concentrations, neurosteroids have the capacity to potentiate GABAergic inhibition on a wider range of GABA_AR subtypes, including synaptic GABA_ARs that mediate the phasic form of GABAergic inhibition (Hosie et al. 2006; Hosie et al. 2009). Further adding to the diversity of neurosteroid actions, GABA_ARs containing the ϵ subunit are relatively insensitive to neurosteroid actions (for a recent review see (Mackenzie and Maguire 2013)). In addition, although neurosteroids are generally considered to be positive allosteric modulators of GABA_ARs, their actions are more diverse. For instance, other prenanolone metabolites including pregnanolone sulfate and DHEAS exhibit inhibitory actions on GABA_ARs and are thought to act via an alternative neurosteroid binding site to that of allopregnanolone (for review see (Carver and Reddy 2013)).

Neurosteroid mediated potentiation of inhibitory tonic GABA_AR mediated currents will have an important influence on neuronal excitability by narrowing the spatial and temporal integration of synaptic events thus increasing the current required to reach action potential threshold (Farrant and Nusser 2005). Due to fluctuations in neurosteroid levels under physiological conditions, the GABA_ARs, must be extremely plastic to maintain an optimal level of inhibition and, thus, neuronal excitability. Thus, in addition to their influence on the biophysical and pharmacological properties of the receptors due to allosteric potentiation and direct activation of the receptors, changes in neurosteroid concentration also influences GABA_AR expression patterns (see section III), possibly by modulating the phosphorylation state of the receptor (Abramian et al. 2010; Kuver et al. 2012). Both increases and decreases in allopregnanolone have been associated with changes in neuronal excitability and mood which are thought to be due in part to the actions of neurosteroids at GABA_ARs making them an attractive therapeutic target. Although it has been suggested that tolerance is less likely to occur with neurosteroid based therapies ((Reddy and Rogawski 2000) commentary by (Lagrange 2006), changes in GABA_AR subunit expression resulting from neurosteroid exposure may alter GABAergic inhibition by altering neurosteroid sensitivity (commentary by (Lagrange 2006). In fact, recent studies suggest neurosteroid tolerance associated with decreased expression of the GABA_AR α_4 subunit (Turkmen et al. 2011) which would have significant implications for the therapeutic potential of neurosteroids. The rest of this review will discuss the changes in GABAergic inhibition evoked by fluctuations in neurosteroid level and the relevance to our understanding of and potential treatment of mood disorders.

III. Regulation of GABA_ARs over the estrous cycle

Changes in GABAergic inhibition over the estrous cycle were originally inferred by the cyclic changes in the binding of, and responses to benzodiazepines, neurosteroids and muscimol (Aldahan et al. 1994; Martin and Williams 1995; Molina-Hernandez et al. 2001; Molina-Hernandez and Tellez-Alcantara 2001; Reddy and Kulkarni 1999; Taherianfard and Mosavi 2011). It has now been shown that during and immediately following periods of elevated progesterone, protein expression of α_4 , β_1 and δ subunits are increased in the periaqueductal grey matter of rats over the estrous cycle (Fig. 1) (Griffiths and Lovick

2005a; Griffiths and Lovick 2005b). Similar results have been obtained in the hippocampus, where during periods of elevated progesterone and progesterone-derived neurosteroids, there is an increase in the expression of the GABA_AR δ subunit (Fig. 1) (Maguire et al. 2005; Wu et al. 2013) and a decrease in the expression of the γ_2 subunit (Maguire et al. 2005). Furthermore, increases in α_4 mRNA and protein expression have also been observed in the hippocampus following progesterone withdrawal (Smith et al. 1998). Progesterone mediated increases in δ subunit expression were blocked by the 5- α reductase inhibitor finasteride (Maguire et al. 2005; Wu et al. 2013) but not by the progesterone receptor inhibitor RU489 (mifepristone, (Maguire et al. 2005)) or in progesterone receptor KO mice (Wu et al. 2013) demonstrating that neurosteroids and not progesterone itself mediate these changes. These results are also mimicked in both progesterone administration and withdrawal models (Griffiths and Lovick 2005a; Gulinello et al. 2001; Hsu and Smith 2003; Shen et al. 2005) demonstrating that sex steroids and their metabolites modify GABAergic inhibition throughout the estrous cycle by altering the expression of specific GABA_AR subunits.

Changes in GABAergic inhibition over the menstrual cycle have been attributed to the development of premenstrual syndrome (PMS) and the more severe premenstrual dysphoric disorder (PMDD), which has just recently been recognized as a distinct mental disorder by psychiatrists in the new Diagnostic and Statistical Manual, the DSM-5 (American Psychiatric Association 2013). The most severe symptoms are reported during the luteal phase when progesterone levels rise and then begin to fall (Lovick 2012). Thus, the pathophysiology of PMS/PMDD is thought to involve a reduction in the levels of neurosteroids. However, although some studies demonstrate alterations in the levels of neurosteroids in PMS/PMDD (Rapkin et al. 1997), many studies do not (for review see (Wihlbäck et al. 2006)). These findings indicate that neurosteroid levels *per se* do not mediate the pathophysiology of PMS/PMDD, but rather the site of action of neurosteroids, namely GABA_ARs, may be altered. Whilst it is not possible to directly measure changes in GABA_AR expression in patients suffering from PMS/PMDD, a change in saccadic eye velocity (SEV) has been used as a proxy measure of GABA_AR activity in different physiological states and following pharmacological challenges (Backstrom et al. 2013). PMS patients have been shown to have a slower SEV compared to controls in the luteal phase (Sundstrom and Backstrom 1998) suggesting differences in the composition of GABA_ARs in the circuitry controlling saccadic eye movements. Furthermore, PMS/PMDD patients are less sensitive to benzodiazepines (Sundstrom et al. 1997a; Sundstrom et al. 1997b) consistent with the hypothesis that GABAergic inhibition is altered in PMS/PMDD patients. PMS patients also show a reduced sensitivity to pregnanolone in the luteal phase (Sundstrom et al. 1998) which may reflect altered regulation of steroidogenic enzymes over the menstrual cycle. Administration of the SSRI fluoxetine reduces symptom severity in PMS/PMDD sufferers possibly by increasing the activity of the steroidogenic enzyme 3 α -HSD and allopregnanolone production independently of the 5HT system (Griffin and Mellon 1999; Lovick 2012; Marjoribanks et al. 2013; Pinna et al. 2009). Increased allopregnanolone production and a more gradual drop in steroid levels in the late luteal phase may help reduce the anxiogenic effects observed following rapid steroid withdrawal (Lovick 2012). In turn it would be anticipated that an increased tonic conductance would also be anxiolytic. Therefore, it is perplexing that negative mood symptoms are reported

during periods when allopregnanolone levels are elevated. However, one would anticipate negative mood symptoms would arise due to deficits or changes in the site of action of neurosteroids (GABA_ARs) despite elevated levels of neurosteroids. Whilst studies in animal models have suggested a role for alterations in GABA_ARs in mood disorders associated with changes in ovarian hormones it remains unclear whether GABA_ARs are actually altered in patients with PMS/PMDD.

One explanation for the anxiogenic actions of neurosteroids over the estrous cycle is that, at low concentrations, neurosteroids preferentially act at inhibitory interneuron populations, reducing their output and increasing the excitability of their target neurons (Backstrom et al. 2013; Lovick 2012; Wang 2011). Indeed, it has been suggested that increased expression of $\alpha_4\beta\delta$ receptors in GABAergic neuronal populations in the PAG following periods of elevated progesterone results in disinhibition of the principal output neurons and increased network excitation (Brack and Lovick 2007; Griffiths and Lovick 2005a; Griffiths and Lovick 2005b; Lovick 2012). However, further increases in neurosteroid levels may dampen network excitability by acting upon the $\gamma 2$ -containing GABA_AR populations which have a lower affinity for neurosteroids creating an inverted U-shaped relationship between steroid concentration and neuronal excitability. This mirrors symptom severity with postmenopausal women who when prescribed progesterone experienced more negative mood symptoms as the serum allopregnanolone concentrations reached levels similar to those measured in the luteal phase (Andreen et al. 2005; Andreen et al. 2006; Wang 2011). However, when allopregnanolone levels were increased further, the severity of reported symptoms decreased (Andreen et al. 2006) with similar responses observed in the aggression levels of rodents (Miczek et al. 2003).

Changes in the neuronal intracellular chloride concentration can switch GABAergic inhibition, and therefore neurosteroid actions, from inhibitory to excitatory which may also contribute to PMS/PMDD symptoms when allopregnanolone levels are elevated (Wang 2011). In the adult, the low intracellular chloride concentration required for GABAergic inhibition is maintained by the potassium chloride co-transporter, KCC2, which sets the GABA reversal potential (E_{GABA}) equal to or below the resting membrane potential (Payne et al. 1996; Payne et al. 2003). However, it is now known that KCC2 surface expression and pumping efficiency is not static but tightly regulated by changes in phosphorylation state (Kahle et al. 2010; Kahle et al. 2013; Lee et al. 2007; Lee et al. 2010; Rinehart et al. 2011; Rivera et al. 2002; Rivera et al. 2004) allowing for quick and reversible changes in chloride concentration. Downregulation of KCC2 and elevated intracellular chloride can lead to a depolarizing shift in E_{GABA} resulting in the excitatory actions of both GABA and neurosteroids. The excitatory actions of neurosteroids due to the potentiation of depolarizing δ -GABA_AR mediated currents are seen in the corticotropin-releasing hormone (CRH) neurons of the paraventricular nucleus (PVN) (Sarkar et al. 2011) where KCC2 is downregulated following an acute stressor (Hewitt et al. 2009; Sarkar et al. 2011). Differences in KCC2 expression within intracellular compartments adds an additional layer of complexity (for review see (Wang 2011) with neurosteroids being shown to potentate excitatory GABAergic currents in the presynaptic terminal contributing to glutamate release (Kim et al. 2011). Although reduced KCC2 function and expression levels can render neurosteroids excitable, changes in KCC2 expression levels over the estrous cycle have yet

to be fully investigated. Interestingly estradiol, which effects mood in a dose dependent manner (Bjorn et al. 2003; Wang 2011), has been shown to potentate the activity of the sodium potassium chloride cotransporter NKCC1 (Nakamura et al. 2004) which, by pumping chloride into the cell, also reduces GABAergic inhibition. However, it is important to remember that sufficiently increasing the GABA_AR mediated conductance, for instance, by elevating the extracellular GABA concentration (or potentially by elevating neurosteroid levels), can overcome the excitatory effects of depolarizing GABA due to shunting inhibition (Song et al. 2011). Therefore, the potential effects of a loss of KCC2 function on neuronal excitability will likely be context specific.

Finally, during puberty neurosteroids have been shown to modulate $\alpha_4\beta\delta$ GABA_AR mediated currents in a polarity dependent way by attenuating hyperpolarizing but not depolarizing responses (Shen et al. 2007; Smith 2013). This neurosteroid dependent reduction in inhibition may also contribute to negative mood symptoms in PMS/PMDD (see section IV. Regulation of GABA_ARs during puberty). These data demonstrate steroid hormone/neurosteroid mediated changes in GABAergic inhibition over the estrous cycle which may contribute to the increased vulnerability to mood disorders during this period and similar deficits may occur under other conditions of altered steroid hormone levels, such as during puberty and pregnancy.

IV. Regulation of GABA_ARs during puberty

Puberty is a period commonly associated with changes in mood, such as increased anxiety and mood swings in addition to being a vulnerable period for the generation of affective disorders (for review see (Smith 2013). Levels of steroid hormones fluctuate during puberty yet the effects of these hormonal changes on neuronal and network excitability during puberty is still relatively understudied. Allopregnanolone levels gradually increase before puberty then drops upon onset (Fadalti et al. 1999; Mannan and O'Shaughnessy 1988; Shen et al. 2007). The fall in allopregnanolone concentrations correspond to a transient increase in $\alpha_4\beta\delta$ expression in the CA1 region of the hippocampus (Shen et al. 2010; Smith et al. 2007) of female mice which are higher than those found in the adult (Shen et al. 2010) and can be mimicked by steroid withdrawal (Smith et al. 2006). Administration of allopregnanolone during puberty has been shown to increase anxiety levels in mice (Shen et al. 2007) whilst having anxiolytic effects when applied to pre-pubertal mice and adults (Bitran et al. 1999; Shen et al. 2007). Although unexpected, the anxiogenic effects of neurosteroids at puberty coincide with a reported increase in the prevalence of anxiogenic effects of benzodiazepines during puberty in human children and adolescents (Massanari et al. 1997). Shen et al (2007) have shown that allopregnanolone can attenuate hyperpolarizing $\alpha_4\beta\delta$ mediated currents in hippocampal CA1 pyramidal neurons by increasing the degree of receptor desensitization. Consequently, the increased $\alpha_4\beta\delta$ receptor desensitization in the presence of allopregnanolone reduced rather than potentiated the magnitude of the tonic conductance resulting in increased neuronal excitability (Shen et al. 2007). Neurosteroids were not anxiogenic in mice lacking the GABA_AR δ subunit (*Gabrd*^{-/-} mice), supporting the role of δ -containing GABA_ARs in mediating the anxiety phenotypes observed during puberty (Shen et al. 2007). In addition to increasing anxiety, the rise in hippocampal $\alpha_4\beta\delta$ expression during puberty and the increase in the tonic conductance have also been shown to impair

learning and memory in the CA1 hippocampus of pubertal mice, an effect which could be rescued by an increase in allopregnanolone (Shen et al. 2010). Therefore, changes in the magnitude of tonic inhibition within the hippocampus are influenced by changes in neurosteroid levels and may provide a novel therapeutic target for managing the development of affective disorders during puberty and adolescence.

V. Regulation of GABA_ARs during pregnancy and the postpartum period

The highest physiological concentrations of neurosteroids are observed during pregnancy. Progesterone levels increase 200-fold (Backstrom et al. 2013) and are paralleled by increases in the neurosteroids allopregnanolone and THDOC (Concas et al. 1998). High levels of neurosteroids have been shown to be essential for a successful pregnancy (Frye et al. 2011) and reach concentrations as high as 100 nM (Paul and Purdy 1992), which is sufficient to cause sedation in non-pregnant animals (Backstrom et al. 2003). Therefore, it has been suggested that plasticity in GABA_ARs occur during pregnancy and the postpartum period as a compensatory mechanism to offset the elevations in neurosteroid levels (for review see (Maguire and Mody 2009)). It has been shown that cortical and hippocampal GABA_AR γ 2 mRNA (Concas et al. 1998; Follesa et al. 1998) and protein (Maguire et al. 2009; Sanna et al. 2009) levels are decreased during pregnancy with no changes observed in α 1, α 2, α 3, α 4, β 1, β 2, β 3 mRNA levels (Concas et al. 1998; Follesa et al. 1998). This downregulation of GABA_ARs was neurosteroid dependent as no changes were observed in the presence of the 5- α reductase inhibitor finasteride (Concas et al. 1998). Furthermore, the GABA_AR δ -subunit is also downregulated during pregnancy in the dentate gyrus, hippocampal CA1, striatum and thalamus (Fig. 1) (Maguire et al. 2009; Maguire and Mody 2008). Interestingly expression levels in the dentate gyrus return to normal 48 hr post partum (Fig. 1) when neurosteroid levels are restored to those observed in virgin animals, suggesting that changes in GABA_AR expression maybe a compensatory mechanism to maintain a steady level of inhibition throughout the pregnancy and postpartum period (Maguire and Mody 2008). Consistent with this hypothesis, allopregnanolone exposure in slices from pregnant mice resulted in comparable levels of network excitability to slices from virgin animals (Maguire et al. 2009). Contrary to the findings in mice, an increase in δ -subunit expression in the dentate gyrus has been observed in rats (Sanna et al. 2009), suggesting that GABA_AR plasticity maybe more complicated than a simple homeostatic mechanism or that species differences exist. However, further supporting compensatory regulation of GABA_ARs during pregnancy, increased expression of the neurosteroid resistant ϵ - subunit has been proposed to play an important role in preventing neurosteroid mediated inhibition of respiratory neurons during pregnancy when neurosteroids levels are particularly high (Hengen et al. 2012).

Changes in neurosteroid concentrations during pregnancy and postpartum have also been shown to effect δ -subunit expression in hippocampal interneuron populations. A reduction in δ subunit expression has been observed in pvalbumin-positive interneurons in the CA3 region of the hippocampus during pregnancy which recovered to normal levels 48hr postpartum (Ferando and Mody 2013). Recently, δ subunit-mediated tonic GABAergic inhibition has been shown to regulate hippocampal interneuron populations (molecular layer and stratum radiatum interneurons) which dramatically controls principal neuron excitability

(Lee and Maguire 2013). Further, it has previously been shown that δ subunit-mediated tonic GABAergic inhibition in pvalbumin positive interneurons contribute to the maintenance of γ -oscillations (Mann and Mody 2010) which are linked to memory formation and cognitive functioning. In the absence of neurosteroids, the reduction in δ -subunit expression in acute slices from pregnant mice corresponded to an increase in γ -frequency oscillations. However, γ -frequency oscillations were normal when slices were exposed to 100 nM allopregnanolone (Ferando and Mody 2013) (Ferando and Mody 2013), neurosteroid levels which are in the physiological range of those observed during pregnancy (Paul and Purdy 1992). This study provides further evidence showing that GABA_AR homeostatic plasticity is essential for maintaining network function at normal levels during periods characterized by elevated levels of neurosteroids, such as pregnancy. As perturbed γ -oscillations are observed in numerous disorders (Uhlhaas and Singer 2010; Uhlhaas and Singer 2012), uncoordinated changes in GABA_AR δ subunit expression and steroid levels may contribute to postpartum depression and other neurological disorders, such as epilepsy and psychosis. Furthermore, changes in γ -frequency oscillations may also arise if unsynchronized changes in δ -subunit expression occur when neurosteroid levels fluctuate over shorter time scales such as during the estrous cycle or stress (Ferando and Mody 2013). These data further support GABA_AR plasticity during pregnancy however, further research is required to fully appreciate the complexity of GABA_AR plasticity during pregnancy and the consequences of failed plasticity, which may contribute to mood disorders associated with pregnancy, such as postpartum depression. Indeed, failure to properly regulate GABA_AR subunit expression during pregnancy and the postpartum period has been suggested to play a role in postpartum depression (Maguire and Mody 2008). Mice which are deficient in the GABA_AR δ subunit (*Gabrd*^{-/-} mice) and, therefore, unable to regulate GABA_AR δ subunit expression throughout pregnancy and the postpartum period, exhibit depression-like behaviors which are restricted to the postpartum period (Maguire and Mody 2008). These data demonstrate that the potential importance of plasticity in GABA_AR subunit expression throughout pregnancy and the postpartum period and suggest that the inability to regulate GABA_ARs, particularly δ subunit-containing receptors, may contribute to the development of postpartum depression.

VI. Conclusions

GABA_AR plasticity has been observed over numerous physiological conditions characterized by changes in neurosteroid levels, including over the estrous cycle, during puberty, and throughout pregnancy and the postpartum period. These alterations in GABA_AR subunit expression likely represent compensatory changes necessary to maintain an ideal level of inhibition in the face of altered neurosteroid levels, which can act on these receptors to potentiate the effects of GABA. A breakdown in the homeostatic plasticity of GABA_ARs under conditions of altered neurosteroid levels may explain the increased vulnerability to mood disorders during this time, such as the manifestation of PMS/PMDD and postpartum depression.

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Figure 1. GABA_AR plasticity over the estrous cycle, puberty, and pregnancy

The diagram summarizes changes in GABA_AR δ subunit expression relative to changes in neurosteroid levels over the estrous cycle (a), puberty (b), and throughout pregnancy and the postpartum period (c). This figure summarizes data obtained from (Griffiths and Lovick 2005a; Griffiths and Lovick 2005b; Maguire et al. 2009; Maguire and Mody 2008; Maguire et al. 2005; Wu et al. 2013).