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Puberty, steroids and GABA_A receptor plasticity

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Summary

GABA_A receptors (GABAR) mediate most inhibition in the CNS and are also a target for neuroactive steroids such as 3 α ,5[α] β -THP (3 α OH-5[α] β -OH-pregnan-20-one or [allo]pregnanolone). Although these steroids robustly enhance current gated by α 1 β 2 δ GABAR, we have shown that 3 α ,5[α] β -THP effects at recombinant α 4 β 2 δ GABAR depend on the direction of Cl⁻ flux, where the steroid increases outward flux, but decreases inward flux through the receptor. This polarity-dependent inhibition of α 4 β 2 δ GABAR resulted from an increase in the rate and extent of rapid desensitization of the receptor, recorded from recombinant receptors expressed in HEK-293 cells with whole cell voltage clamp techniques. This inhibitory effect of 3 α ,5[α] β -THP was not observed at other receptor subtypes, suggesting it was selective for α 4 β 2 δ GABAR. Furthermore, it was prevented by a selective mutation of basic residue arginine 353 in the intracellular loop of the receptor, suggesting that this might be a putative chloride modulatory site. Expression of α 4 β 2 δ GABAR increases markedly at extrasynaptic sites at the onset of puberty in female mice. At this time, 3 α ,5[α] β -THP decreased the inhibitory tonic current, recorded with perforated patch techniques to maintain the physiological Cl⁻ gradient. By decreasing this shunting inhibition, 3 α ,5[α] β -THP increased the excitability of CA1 hippocampal pyramidal cells at puberty. These effects of the steroid were opposite to those observed before puberty when 3 α ,5[α] β -THP reduced neuronal excitability as a pre-synaptic effect. Behaviorally, the excitatory effect of 3 α ,5[α] β -THP was reflected as an increase in anxiety at the onset of puberty in female mice. Taken together, these findings suggest that the emergence of α 4 β 2 δ GABAR at the onset of puberty reverses the effect of a stress steroid. These findings may be relevant for the mood swings and increased response to stressful events reported in adolescence.

Keywords

pregnanolone; allopregnanolone; puberty; GABA-A receptor; neurosteroid; stress; chloride; anxiety; tonic current; CA1 hippocampus; steroid withdrawal

Introduction

It is well-known that the onset of puberty can be associated with mood swings (Buchanan et al., 1992; Huerta and Brizuela-Gamino, 2002) and irritability (Hayward and Sanborn, 2002), in contrast to pre-pubertal behavior. There is an increased response to stress (Modesti et al.,

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2006) at this time, and anxiety disorders, including panic disorder, typically first present at pubertal ages (Hayward and Sanborn, 2002), more likely to occur in girls than boys. There are also reports of increases in risk-taking behavior (Costello et al., 2007), including an increased probability of consumption of addictive substances. Although mood changes associated with premenstrual syndrome (Halbreich et al., 2007), postpartum depression and post-menopausal dysphoria (Mukai et al., 2008) have been investigated in clinical and pre-clinical studies, relatively little is known about the underlying CNS mechanisms which precipitate mood changes at puberty.

In females, the onset of reproductive puberty (gonadarche, menarche) is preceded by maturation of the adrenal gland (adrenarche) (Apter and Hermanson, 2002). Therefore, fluctuations in both adrenal (progesterone, androgens) and gonadal hormones (estradiol) are associated with female pubertal maturation in humans and rodents. Specifically, circulating levels of the steroid hormones progesterone and estradiol rise from very low levels earlier in development to peak immediately preceding the onset of puberty (Apter and Hermanson, 2002; Kahn et al., 2008; Safranski et al., 1993), defined in humans as the onset of menstruation and in female rodents as the time of vaginal opening. Steroid levels decline thereafter (Kahn et al., 2008; Fadalti et al., 1999; Shen et al., 2007) until ovarian cyclicity begins days later. It is this decline in steroid hormones which resembles the hormonal events associated with other hormonally-associated mood syndromes, such as premenstrual syndrome (premenstrual dysphoric disorder or PMDD) and post-menopausal dysphoria (Halbreich et al., 2007). Although ovarian hormones have been reported to exert an array of effects on mood and behavior, effects of neuroactive steroids on the GABA_A receptor (GABAR) may play a role in pubertal anxiety disorders.

The GABA_A receptor

The GABAR is a pentameric membrane protein that mediates most inhibition in the brain (Hevers and Luddens, 1998; Olsen and Sieghart, 2008), and is the target of most anxiety-reducing, sedative drugs, including benzodiazepines, barbiturates, anesthetics and, in some cases, alcohol (Sundstrom-Poromaa et al., 2002; Wallner, et al., 2003; Wei et al., 2004; Glykys et al., 2007). The GABAR plays a pivotal role in regulating anxiety, as demonstrated by studies using transgenic mouse models (Rudolph et al., 1999). This receptor is typically composed of 2 α , 2 β and a γ subunit (Chang et al., 1990), but other subunits exist, including δ , ϵ , π , ρ and θ . Many subunit subtypes also exist (6 α , 3 β , 3 γ) yielding a diverse array of possible GABAR isoforms (Hevers and Luddens, 1998; Olsen and Sieghart, 2008), with different biophysical and pharmacological properties that can differentially alter circuit properties in limbic structures and ultimately have an impact on behavior. Each subunit, in turn, is composed of 4 membrane-spanning α -helices, where TM2 surrounds a central Cl⁻ channel and the TM3-TM4 intracellular loop is a site for phosphorylation. GABA agonists bind at two specific sites between α and β to gate a Cl⁻ current, which can be modulated by a variety of agents, including steroids (Majewska et al., 1986), at distinct sites separate from GABA (Hosie et al., 2006). The most commonly expressed GABAR is $\alpha 1\beta 2\gamma 2$. In contrast, GABAR of the form $\alpha 4\beta \delta$ have relatively low expression in the brain (Wisden et al., 1992), but instead are remarkably plastic, increasing in response to hormonal fluctuations, either endogenously occurring across the ovarian cycle (Lovick et al., 2005; Maguire et al., 2005) or pregnancy (Maguire and Mody, 2009; Sanna et al., 2009) or exogenously administered (Griffiths and Lovick, 2005; Maguire and Mody, 2007; Shen et al., 2005; Smith et al., 1998a; Sundstrom-Poromaa et al., 2002). In particular, withdrawal from chronic administration of progesterone increases expression of $\alpha 4\beta \delta$ in CA1 hippocampus (Smith et al., 2006; Sundstrom-Poromaa et al., 2002), an area which normally has very low expression of this receptor.

$\alpha 4\beta\delta$ GABAR

The $\alpha 4\beta 3\delta$ GABAR is composed of 2 α , 2 β and 1 δ subunit (Barrera et al., 2008), arranged as $\alpha\beta\alpha\delta\beta$, counter-clockwise when viewed from the extracellular space. δ -containing GABAR are relatively unique in that they are not localized to postsynaptic sites, but rather are extrasynaptic or peri-synaptic (Wei et al., 2003) where they generate a tonic current (Stell and Mody, 2002) in response to ambient concentrations of GABA regulated by GABA transporters (Wu et al., 2001) and spillover from synaptic release (Glykys and Mody, 2007). Recombinant δ -containing GABAR have a high sensitivity to low concentrations of GABA (Brown et al., 2002; Sundstrom-Poromaa et al., 2002) and little desensitization (Bianchi et al., 2002; Brown et al., 2002; Feng et al., 2006; Haas and Macdonald, 1999), making them ideally suited as an extrasynaptic receptor. They also possess a unique pharmacological profile, in that they are insensitive to benzodiazepine modulation (Brown et al., 2002), respond with high sensitivity to low concentrations of the GABA agonist gaboxadol (THIP) (Brown et al., 2002) and appear to be the most sensitive targets of steroid modulation (Belelli et al., 2002; Bianchi and Macdonald, 2003; Brown et al., 2002; Wohlfarth et al., 2002; Zheleznova et al., 2008). Specifically, steroids such as $3\alpha,5[\alpha]\beta$ -THP (3α -OH-5 $[\alpha]\beta$ -pregnan-20-one or [allo] pregnanolone) and THDOC are potent positive modulators of recombinant δ -containing GABAR at physiological concentrations (10-30 nM), where they increase receptor efficacy (Bianchi and Macdonald, 2003; Zheleznova et al., 2008).

Although $\alpha 4\beta\delta$ and the homologous $\alpha 6\beta\delta$ GABAR have relatively low expression in most areas of the brain (Wisden et al., 1992), high levels of expression in cerebellar granule cells, dentate gyrus granule cells and thalamic relay nuclei generate a tonic inhibitory current (Belelli et al., 2005; Brickley et al., 2001; Herd et al., 2007; Jia et al., 2005; Mtchedlishvili and Kapur, 2006; Stell and Mody, 2002), while the tonic inhibitory current in CA1 hippocampus (Bai et al., 2000) is primarily mediated by $\alpha 5$ -containing GABAR (Caraiscos et al., 2004). Tonic current mediated by native δ -containing GABAR is highly responsive to low concentrations of gaboxadol, similar to recombinant δ -containing GABAR. In addition, a number of reports have also shown significant modulation of the tonic current by 10 nM concentrations of neurosteroids such as THP and THDOC in dentate gyrus granule cells (Stell et al., 2003; Maguire and Mody 2003; Scimeni et al., 2006; Liang et al., 2008; Sanna et al., 2009; Spigelman et al., 2003). However, other studies describe no or only modest effects of steroids on native δ -containing GABAR (Belelli and Herd, 2003; Hamann et al., 2002; Mtchedlishvili and Kapur, 2006) even at higher 250 nM concentrations (Porcello et al., 2003). These conflicting findings may be due to age, species or gender differences or may be the result of other factors such as phosphorylation state (Fanscik et al., 2002; Harney et al., 2003), local neurosteroid metabolism (Belelli and Herd, 2003) or the ambient GABA level which could contribute to the ultimate effect of steroids on tonic current mediated by δ -containing GABAR.

The neurosteroid $3\alpha,5[\alpha]\beta$ -THP

$3\alpha,5[\alpha]\beta$ -THP is formed via two enzymatic conversions from the ovarian/adrenal steroid progesterone (Mellon and Vaudry, 2001), and its levels fluctuate across the ovarian cycle and pregnancy. This steroid can also be formed directly from cholesterol in certain neurons, such as the CA1 hippocampal pyramidal cell (Agis-Balboa et al., 2006), via side chain cleavage enzyme. Thus, it is classified both as a neurosteroid (formed in the brain) as well as a neuroactive steroid (alters brain activity). Neurosteroid synthesis has been shown to be dramatically increased by stress (Purdy et al., 1991; Barbaccia et al., 1996). In particular, 20-45 min of restraint stress significantly increases CNS levels of $3\alpha,5[\alpha]\beta$ -THP 20-fold in rodents (Higashi et al., 2005; Mukai et al., 2008), while human studies have indicated that performance stress increases circulating levels of $3\alpha,5[\alpha]\beta$ -THP (Girdler et al., 2006; Droogelever et al., 2004).

Behaviorally, $3\alpha,5[\alpha]\beta$ -THP has been shown to reduce anxiety (Bitran et al., 1999) and possess anti-convulsant effects (Frye, 1995) in rodents and, at high doses, has sedative effects in both rodents (Lancel et al., 1997) and humans (van Broekhoven et al., 2006). In particular, the anxiety-reducing effects of this steroid have been demonstrated via systemic injection or direct infusion into the dorsal hippocampus (Bitran et al., 1999) in female rats. Thus, stress-induced increases in the steroid may serve as a compensatory response, because the resultant increase in GABAergic inhibition by the steroid would permit an adaptive decrease in the stress-induced anxiety response.

Puberty and $\alpha 4\beta\delta$ GABAR

Because responses to stress are increased at the onset of puberty (Modesti et al., 2006), we examined whether steroid/GABAR interactions were altered during this time in female mice as a result of changes in expression of their most sensitive target, $\alpha 4\beta\delta$ GABAR. To this end, we initially examined the expression of $\alpha 4$ and δ GABAR subunits in CA1 hippocampus, comparing results from female C57BL6 mice before puberty onset with those shortly after vaginal opening, the onset of puberty. In fact, hippocampal expression of both $\alpha 4$ and δ subunits was increased by 2-3-fold at the onset of puberty, assessed using Western blot techniques, compared to levels before puberty (Shen et al., 2007). In addition, immunocytochemical procedures revealed a striking increase in expression of both subunits along the apical dendrites of CA1 pyramidal cells compared to almost undetectable levels prior to puberty. Because δ -containing GABAR are highly sensitive to the GABA agonist gaboxadol (THIP) (Brown et al., 2002), we examined responses of CA1 hippocampal pyramidal cells to this drug using whole cell patch clamp recording in the hippocampal slice. In fact, gaboxadol application produced a significantly greater shift in the holding potential in pubertal slices compared to its effect before puberty (Shen et al., 2007). These results suggest that there is an increase in functional $\alpha 4\beta\delta$ GABAR at puberty.

Consistent with an increase in inhibitory tone at puberty, spontaneous spiking of CA1 pyramidal cells was decreased at this time (Shen et al., 2007) compared to pre-pubertal neuronal activity levels, assessed using cell-attached recording techniques. Taken together, these results suggest that puberty onset is a time of decreased levels of activity in limbic structures, which does not explain the increase in anxiety reported at this time (Buchanan et al., 1992; Hayward and Sanborn, 2002; Modesti et al., 2006).

$3\alpha,5[\alpha]\beta$ -THP effects at puberty

In order to investigate possible reasons for increased anxiety at puberty, we tested the hypothesis that the change in GABAR expression would alter responses to the stress steroid $3\alpha,5[\alpha]\beta$ -THP. This hypothesis was justified, in part, by clinical reports suggesting that mood changes in women with premenstrual dysphoric disorder were due to an abnormal response to progesterone (Schmidt et al., 1998), the precursor of $3\alpha,5[\alpha]\beta$ -THP. In addition several reports have indicated that in women with PMDD (Freeman et al., 2002) and post-menopausal dysphoria (Andreen et al., 2004), worsened mood was paradoxically associated with higher circulating levels of $3\alpha,5[\alpha]\beta$ -THP. These reports suggest the possibility that hormonally induced changes in mood might be the result of a negative response to $3\alpha,5[\alpha]\beta$ -THP.

When tested using the cell-attached recording paradigm, $3\alpha,5[\alpha]\beta$ -THP indeed produced the opposite effects at puberty (Shen et al., 2007), increasing spontaneous spiking of CA1 pyramidal cells, in contrast to its inhibitory effect before puberty. This excitatory effect of the GABA-modulatory steroid was not seen in pyramidal cells from δ -knock out mice, suggesting that effects at the $\alpha 4\beta\delta$ GABAR might be a likely target for this effect.

3 α ,5[α] β -THP effects at α 4 β 2 δ GABAR

Numerous studies using recombinant δ -containing GABAR have demonstrated that this receptor is a highly sensitive target for steroids such as 3 α ,5[α] β -THP and THDOC (Belelli et al., 2002; Brown et al., 2002; Wohlfarth et al., 2002; Zheleznova et al., 2008), which normally increases GABA-gated current. However, most conventional recording is generally performed by assessing the outward flux of Cl⁻ ions, while in many areas of the mature CNS, Cl⁻ flux is inward (outward current) (Rivera et al., 1999). Thus, we tested the possibility that varying the direction of Cl⁻ flux might have an impact on the effect of 3 α ,5[α] β -THP at recombinant α 4 β 2 δ GABAR, expressed in HEK-293 cells. As expected, 30 nM 3 α ,5[α] β -THP produced robust increases in current gated by either α 1 β 2 δ or α 4 β 2 δ when Cl⁻ flux was outward (Fig. 1). However, in a separate set of cells, 3 α ,5[α] β -THP decreased GABA-gated current under conditions of inward Cl⁻ flux at α 4 β 2 δ GABAR (Shen et al., 2007), but not at α 1 β 2 δ receptors (Fig. 1). Substituting β 3 for β 2 yielded a receptor that was not inhibited by 3 α ,5[α] β -THP, nor was the steroid effective at inhibiting α 4 β 2 γ 2, α 1 β 2 γ 2 or α 5 β 3 γ 2, suggesting that α 4 β 2 δ was uniquely sensitive to 3 α ,5[α] β -THP inhibition when Cl⁻ flux was inward. This is the likely receptor combination expressed endogenously, as suggested by studies conducted using the GABAR β 2 knock-out mouse (Belelli et al., 2005; Herd et al., 2007), which reduces the tonic current in both dentate gyrus and thalamus, two areas with high levels of α 4 and δ subunit expression (Wisden et al., 1992).

3 α ,5[α] β -THP effects on desensitization of α 4 β 2 δ receptors

3 α ,5[α] β -THP did not change the reversal potential at α 4 β 2 δ receptors, suggesting that it did not alter conductances other than those generated by GABA, and its inhibitory effect increased with increasing concentrations of GABA (Shen et al., 2007), suggesting that it might attenuate current by increasing receptor desensitization. In fact, when tested directly using rapid agonist application, 3 α ,5[α] β -THP produced a significantly faster rate of desensitization and increased the extent of desensitization from 8 to 87%, when Cl⁻ flux was inward (Fig. 1, Shen et al., 2007). In contrast, 3 α ,5[α] β -THP did not increase desensitization with outward Cl⁻ flux, but, in fact, increased the peak current, suggesting that it increased receptor efficacy, as has been reported previously (Bianchi and Macdonald, 2003). Previous studies have suggested that steroids such as THDOC increase desensitization of homologous α 6 β 2 δ GABAR (Bianchi et al., 2002; Haas and Macdonald, 1999), which exhibits a faster desensitization when Cl⁻ flux is inward (Bianchi et al., 2002). In contrast, α 5-containing GABAR, which typically constitute the majority of extrasynaptic GABAR in CA1 hippocampus, desensitize more quickly with outward Cl⁻ flux (Burgard et al., 1996).

Mutagenesis studies

In order to determine a potential molecular mechanism for this polarity-dependent inhibition of α 4 β 2 δ GABAR by 3 α ,5[α] β -THP, it was first necessary to compare sequence homologies of α 1 and α 4, as α 1 β 2 δ GABARs are not sensitive to 3 α ,5[α] β -THP inhibition as are α 4 β 2 δ GABARs. The least homologous region of these subunits is the intracellular TM3-TM4 loop (Fig. 2), which is twice as long in α 4, and possesses only a 10% sequence homology with the intracellular TM3-TM4 loop in α 1 (Shen et al., 2007). In addition there are 16 unique positively charged residues in the α 4 loop which are not present in α 1, the most likely target for an effect dependent upon the negatively charged Cl⁻ ion. Site-directed mutagenesis of these individual residues revealed that arginine 353 in the loop, when mutated to a neutral glutamine, prevented the 3 α ,5[α] β -THP inhibition of α 4[R353Q] β 2 δ GABAR (Fig 2). This mutation did not change the concentration-response curve or current-voltage relationship, suggesting that it did not alter other conductances. In contrast, mutation of a nearby arginine (351) in α 4 did not prevent 3 α ,

5[α] β -THP inhibition of $\alpha 4[R351Q]\beta 2\delta$, suggesting that arginine 353 alone may be a putative Cl⁻ modulatory site.

Modulatory effects of ions

Modulatory effects of Cl⁻ have been noted before (Lo and Snyder, 1983; Olsen and Snowman, 1982), which are necessary for barbiturate and benzodiazepine enhancement of GABA binding. Recent studies suggest that ion sensor sites can regulate other events such as Cl⁻ activation of HCN subunits which mediate I_h (Chen et al., 2000). In addition, the recent discovery (Ramsey et al., 2006) of a cation-triggered phosphorylation event in a novel membrane protein lacking an ion pore suggests that ion sensor sites regulate neuronal function beyond ion conductance. In addition, the intracellular loop of the Cys-loop family of receptors is ion accessible (Kelley et al., 2003; Miyazawa et al., 2003), while for other membrane receptors this loop functions not only as a permeation pathway, but also as a site necessary for rapid desensitization (Turner and Raymond, 2005).

Tonic current

Because $\alpha 4\beta 2\delta$ GABAR are increased at the onset of puberty, 3 $\alpha, 5[\alpha]\beta$ -THP effects were tested on the tonic GABAergic current at this time. Initially, the polarity of the GABAergic current was verified using tight seal cell-attached recording (Perkins, 2006), which reflected an outward current (inward Cl⁻ flux) (Shen et al., 2007). Then, 3 $\alpha, 5[\alpha]\beta$ -THP effects on the tonic current were tested using gramicidin perforated patch recording techniques to maintain the internal Cl⁻ milieu. Recordings were carried out in the presence of TTX, kynurenic acid and 1 μ M GABA to isolate the postsynaptic GABAergic response. Under these conditions, application of 30 nM 3 $\alpha, 5[\alpha]\beta$ -THP reduced the GABAergic current in slices from pubertal mice (Shen et al., 2007), but was without effect in slices from pre-pubertal animals. This effect of the steroid reversed when recordings were carried out in whole cell mode under conditions of outward Cl⁻ flux, suggesting a polarity-dependent effect. In contrast, 3 $\alpha, 5[\alpha]\beta$ -THP had no effect on the tonic current recorded from δ knock-out mice recorded at puberty, consistent with an effect at $\alpha 4\beta 2\delta$ GABAR. In addition, 3 $\alpha, 5[\alpha]\beta$ -THP did not alter the reversal potential, suggesting that it was targeting GABA-gated current alone. In contrast to its effect on the tonic current, 3 $\alpha, 5[\alpha]\beta$ -THP had no effect on synaptic current recorded at the onset of puberty, where sIPSC amplitude, frequency and τ were unchanged by steroid treatment.

3 $\alpha, 5[\alpha]\beta$ -THP effects on neuronal excitability

The functional impact of increased extrasynaptic GABARs along the dendrites at puberty was to reduce the input resistance of the neuron, assessed as the current response to a 10 mV step (Shen et al., 2007). By decreasing this tonic current, 3 $\alpha, 5[\alpha]\beta$ -THP increased the input resistance of the neurons. Thus, 3 $\alpha, 5[\alpha]\beta$ -THP would make the neuron more responsive to depolarizing current at puberty. This was tested directly by assessing the threshold for evoking an action potential in response to depolarizing current, recorded with whole cell current clamp techniques from CA1 hippocampal pyramidal cells in the slice (Shen et al., 2007). In fact, 3 $\alpha, 5[\alpha]\beta$ -THP reduced the threshold for triggering spiking and increased the number of spikes, consistent with its effect to reduce tonic inhibition at puberty (Fig. 3). This is in contrast to its effect before puberty, when it reduced spiking, and in the δ knock-out mouse where it had no effect, suggesting that the emergence of $\alpha 4\beta 2\delta$ GABAR at puberty reverses the effect of this steroid to an excitatory role.

3 $\alpha, 5[\alpha]\beta$ -THP effects on anxiety

The reversal in 3 $\alpha, 5[\alpha]\beta$ -THP effects at puberty was also reflected behaviorally, where 3 $\alpha, 5[\alpha]\beta$ -THP increased anxiety in pubertal female mice (Shen et al., 2007), in contrast to its typical

anxiety reducing effect. Anxiety was tested using the elevated plus maze, a device elevated 3 feet above the floor, which assesses the preference of a rodent for the open versus the closed arms of the maze. A decrease in time spent in the open arm of the maze reflects an increase in anxiety (Pellow et al., 1995). In fact, administration of 10 mg/kg $3\alpha,5[\alpha]\beta$ -THP, i.p., significantly reduced the open arm time at puberty, reflecting an increase in anxiety, in contrast to its effect in pre-pubertal mice, where it significantly increased open arm time, reflecting a decrease in anxiety, as has been shown previously in adult rodents (Akwa et al., 1999; Bitran et al., 1993; Bitran et al., 1999; Frye et al., 2009).

Stress effects at puberty

$3\alpha,5[\alpha]\beta$ -THP is not only a metabolite of the ovarian/adrenal hormone progesterone, but is also released in response to stress (Purdy et al., 1991; Barbaccia et al., 1996; Girdler et al., 2006; Drooglever et al., 2004). 45 min of restraint stress produce maximal CNS levels of this steroid in rodents (Higashi et al., 2005; Mukai et al., 2008). When tested 20 min. after this stress paradigm, pubertal mice decreased their time in the open arm of the maze (Shen et al., 2007), reflecting an increase in anxiety, again, in contrast to the pre-pubertal, as well as adult, female mice. Stress is a complex behavioral state, with multiple sequelae, including rapid increases in sympathetic tone (Goldstein and Kopin, 2008), with ensuing increases in cardiovascular and pulmonary function. Corticosterone is also released by stress and has multiple effects (Goldstein and Kopin, 2008), which include long-term changes in hippocampal neuronal function (McEwen, 2005). Therefore, in order to assess the role of $3\alpha,5[\alpha]\beta$ -THP in producing anxiety in pubertal mice, we pre-administered finasteride, a 5α -reductase inhibitor, to reduce stress-induced increases in $3\alpha,5[\alpha]\beta$ -THP levels. This enzyme inhibitor has been shown to effectively reduce $3\alpha,5[\alpha]\beta$ -THP levels produced by stress to almost undetectable (Mukai et al., 2008), as a dose-dependent effect. In fact, pre-administration of finasteride prevented the effect of stress on this behavioral outcome, in both pre- and pubertal mice (Shen et al., 2007). In addition, pre-administration of the inactive 3β -OH-THP isomer (Lundgren et al., 2003) prevented the anxiety-producing effect of $3\alpha,5[\alpha]\beta$ -THP, suggesting that alterations in anxiety level produced by sustained exposure to a behavioral stressor are mediated by $3\alpha,5\alpha$ -THP release. These results suggest that increases in $3\alpha,5\alpha$ -THP levels produced by stress increase anxiety at puberty, thus amplifying the stress response. In contrast, this steroid reduces anxiety in adults, consistent with results demonstrating $3\alpha,5[\alpha]\beta$ -THP-induced reductions in corticosterone levels in adult rodents (Guo et al., 1995).

Because the inhibitory effect of $3\alpha,5[\alpha]\beta$ -THP was selective for $\alpha 4\beta 2\delta$ GABAR, we examined effects of restraint stress in mice lacking expression of the δ GABAR subunit. In fact, restraint stress did not alter anxiety level in δ knock-out mice at puberty (Shen et al., 2007), implicating δ -containing GABAR as the target for this paradoxical effect of the steroid. Taken together, these results suggest that the increase in expression of $\alpha 4\beta \delta$ GABAR at puberty reverses the effect of a stress steroid such that it reduces inhibition, thereby increasing anxiety. These results may be important for the increase in anxiety and mood swings reported at puberty.

Steroid withdrawal and δ -containing GABAR

In addition to puberty, hormonal fluctuations, either endogenous or exogenously administered, have also been shown to increase expression of the δ subunit in CNS areas (Lovick et al., 2005; Maguire and Mody, 2007; Sundstrom-Poromaa et al., 2002) such as CA1 hippocampus, dentate gyrus and the periaqueductal gray, although the effective timecourse varies in a regional and species-dependent manner. Short-term exposure to neurosteroids (Maguire and Mody, 2007; Shen et al., 2005), such as $3\alpha,5[\alpha]\beta$ -THP or THDOC, from 30 min. to 48 h, increases expression of the δ subunit, in some cases in association with increased expression of the $\alpha 4$ subunit. These increases in δ expression were also associated with an increased responsiveness

of the tonic inhibitory current to low concentrations of the GABA agonist gaboxadol, pharmacologically consistent with increased expression of functional δ -containing GABAR (Brown et al., 2002). In contrast, withdrawal from elevated circulating levels of $3\alpha,5[\alpha]\beta$ -THP also increase expression of δ subunit, in some cases, along with increased expression of the $\alpha 4$ subunit, in CA1 hippocampus (Shen et al., 2007), the periaqueductal gray (Griffiths and Lovick, 2005) and the dentate gyrus following pregnancy-induced elevations in steroids such as $3\alpha,5[\alpha]\beta$ -THP (Maguire and Mody, 2009).

Steroid withdrawal

Steroid withdrawal in rats is produced by cessation of chronic exogenous treatment (Smith et al., 1998a). However, steroid withdrawal in mice is accomplished using a 5α -reductase blocker (Smith et al., 2006) to block the normally high levels of this steroid. Mice exhibit circadian fluctuations in circulating levels of $3\alpha,5\alpha$ -THP (Corpechot et al., 1997), as recently also described in humans at puberty (McCartney and et al., 2007), with levels which peak at high 30 nM concentrations in the early hours of the active phase (dark for rodents, light for humans). (Unlike humans, mice only produce $3\alpha,5\alpha$ -THP but not $3\alpha,5\beta$ -THP.) Therefore, a withdrawal state can easily be induced in mice using a 5α -reductase blocker such as finasteride to prevent formation of $3\alpha,5\alpha$ -THP during its nocturnal surge (Smith et al., 2006).

$3\alpha,5\alpha$ -THP withdrawal

In fact, $3\alpha,5\alpha$ -THP withdrawal undertaken in pre-pubertal female mice produced increases in sensitivity of the tonic inhibitory current to application of gaboxadol (Shen et al., 2007) and inhibition by the trivalent cation lanthanum, results pharmacologically consistent with increased expression of $\alpha 4\beta\delta$ GABAR (Brown et al., 2002; Saxena et al., 1997), similar to findings at puberty (Shen et al., 2007). $3\alpha,5[\alpha]\beta$ -THP administration also reduced the outward tonic current, and increased spiking assessed in cell attached mode following $3\alpha,5\alpha$ -THP withdrawal, in a manner similar to its effect at puberty (Shen et al., 2007). In addition, $3\alpha,5\alpha$ -THP withdrawal resulted in a state where acute in vivo administration of $3\alpha,5[\alpha]\beta$ -THP triggered anxiety, also similar to its effect at puberty (Shen et al., 2007).

Replacement $3\alpha,5[\alpha]\beta$ -THP and puberty

Interestingly, puberty was also associated with a decline ("withdrawal") in $3\alpha,5\alpha$ -THP (Shen et al., 2007) of similar magnitude to that seen with finasteride injection (Palumbo et al., 1995; Smith et al., 2006), well-correlated with the decline in progesterone which has been reported (Kahn et al., 2008). Thus, the increase in $\alpha 4\beta\delta$ GABAR expression may represent a compensatory CNS mechanism to normalize the level inhibition in response to this decline in a GABA-enhancing steroid.

In order to test whether the decline in $3\alpha,5\alpha$ -THP at the onset of puberty triggered the increase in $\alpha 4$ and δ expression, we administered replacement $3\alpha,5[\alpha]\beta$ -THP to pubertal mice across a 48 h period. This treatment normalized levels of $\alpha 4$ and δ expression to control levels and reduced the response of the tonic current to gaboxadol (Shen et al., 2007), suggesting that expression of functional $\alpha 4\beta\delta$ GABAR was reduced to control levels. By normalizing the GABAR population, replacement $3\alpha,5[\alpha]\beta$ -THP restored the inhibitory effect of the steroid, which reduced spontaneous spiking of CA1 pyramidal cells. This normalizing effect of replacement $3\alpha,5[\alpha]\beta$ -THP was also evidenced behaviorally (Shen et al., 2007), where restraint stress resulted in decreased anxiety, similar to its effect in control pre-pubertal female mice. Thus, these findings suggest that the increase in $\alpha 4\beta\delta$ GABAR at puberty is a direct result of the declining levels of $3\alpha,5\alpha$ -THP seen at this time.

Premenstrual dysphoric disorder

The $3\alpha,5\alpha$ -THP withdrawal state in mice may also be relevant as a rodent model for premenstrual dysphoric disorder (PMDD), another hormonal transition state associated with mood disturbances, especially irritability, anxiety and depression (Endicott et al., 1999; Halbreich et al., 2007; Yonkers, 1997). In women with PMDD, dysphoric mood follows a period of declining levels of progesterone and $3\alpha,5[\alpha]\beta$ -THP (the follicular phase), but can frequently begin sometime after the onset of the luteal phase, when progesterone and $3\alpha,5[\alpha]\beta$ -THP levels are increasing. Recent studies (Freeman et al., 2002; Schmidt et al., 1998) have suggested that women with PMDD may have an atypical response to progestins, such as $3\alpha,5[\alpha]\beta$ -THP. Similarly, female mice undergoing $3\alpha,5[\alpha]\beta$ -THP withdrawal respond in an atypical manner to acute application of $3\alpha,5[\alpha]\beta$ -THP (Smith et al., 2006), where it increases anxiety in the elevated plus maze, when preceded by a brief electrical shock. In contrast, $3\alpha,5[\alpha]\beta$ -THP decreases anxiety in female mice not undergoing steroid withdrawal (Akwa et al., 1999; Bitran et al., 1993; Bitran et al., 1999; Frye et al., 2009; Smith et al., 2006). However, a recent study (Kask et al., 2009) has suggested that $3\alpha,5\alpha$ -THP does not produce different effects in women with PMDD, suggesting that other factors play a role in this disorder. Alternatively, the effect of $3\alpha,5\alpha$ -THP may vary across the luteal phase when progestin levels are fluctuating, or as suggested in the rodent model (Smith et al., 2006), may only be seen when adverse mood is triggered by an external event.

Behavioral pharmacology and $\alpha 4\beta\delta$ GABAR

Because the increased expression of $\alpha 4\beta\delta$ GABAR following $3\alpha,5\alpha$ -THP withdrawal would alter GABAR pharmacology (Brown et al., 2002), the behavioral effects of a variety of drugs which are active at GABAR were tested for their effect in altering behavior on the plus maze. $\alpha 4\beta\delta$ GABAR are insensitive to modulation by conventional benzodiazepine agonists (such as lorazepam) (Brown et al., 2002), but have greater sensitivity to the GABA agonist gaboxadol (THIP) (Brown et al., 2002) and are inhibited by low concentrations of the benzodiazepine antagonist flumazenil (Dunn et al., 2003). In fact, these drugs differentially altered open arm time in controls and $3\alpha,5\alpha$ -THP withdrawn mice in a manner consistent with upregulation of $\alpha 4\beta\delta$ GABAR after steroid withdrawal; that is, following $3\alpha,5\alpha$ -THP withdrawal, lorazepam was ineffective in increasing open arm time (Smith, 2006), which is its typical effect in control animals. In addition, gaboxadol produced a greater increase in open arm time after $3\alpha,5\alpha$ -THP withdrawal than in controls, while a low dose of flumazenil (2 mg/kg) decreased open arm time, suggesting that it increased anxiety after $3\alpha,5\alpha$ -THP withdrawal, but was ineffective in vehicle-injected controls. These pharmacological findings are in fact also consistent with clinical reports of women with PMDD, who exhibit a relative benzodiazepine insensitivity (Sundstrom et al., 1997), assessed by mood ratings as well as by eye saccade velocity, an objective measure of GABAergic tone. In addition, flumazenil precipitates panic attacks in women with PMDD (Le Melleo et al., 2000), but not in normal control subjects. Taken together, these findings suggest that $3\alpha,5\alpha$ -THP withdrawal in the female mouse may represent a useful model of PMDD.

Progesterone withdrawal and the periaqueductal gray

Recent studies in the rat have demonstrated that withdrawal from progesterone, as well as naturally occurring hormonal fluctuations across the ovarian cycle increase $\alpha 4$ and δ subunit expression in the periaqueductal gray, an area with a direct role in mediating the panic response (Lovick, 2000). The activity of the output neurons in this area is under the control of GABAergic inhibition. Recent studies (Lovick et al., 2005) have demonstrated that fluctuations in endogenous steroids across the estrous cycle alter the expression of GABAR subunit combinations in this region: $\alpha 4$, $\beta 1$ and δ subunit expression was increased on PAG

interneurons on the day of late diestrus. This stage of the cycle follows the secondary peak in progesterone and $3\alpha,5\alpha$ -THP which occurs on early diestrus, and could thus be considered a time of $3\alpha,5\alpha$ -THP “withdrawal”. In fact, the authors have a second study demonstrating similar increases in $\alpha 4$, $\beta 1$ and δ GABAR subunit expression following withdrawal from administered progesterone (Griffiths and Lovick, 2005). Because this increase in GABAR expression is on the inhibitory interneurons, it would effectively “disinhibit” the output neurons, as has recently been shown. In this case, both application of bicuculline as well as the panicogenic cholecystokinin ligand pentagastrin increased PAG neuronal activity to a greater extent on estrus and late diestrus (Brack and Lovick, 2006), suggesting a reduced GABAergic tone on the output neurons on these two days of $3\alpha,5\alpha$ -THP “withdrawal” across the estrous cycle. Activation of these PAG output neurons has been shown to elicit a panic reaction in both humans and rodents (Lovick, 2000), because it is part of an afferent pathway to brainstem respiratory neurons that control the rate and amplitude of respiratory signals (Voituron et al., 2005). These findings have important clinical significance as well because many women with panic disorder exhibit premenstrual exacerbation during the decline in progesterone at the end of the luteal phase (Yonkers, 1997) (i.e., a $3\alpha,5[\alpha]\beta$ -THP “withdrawal” state).

Steroid withdrawal and $\alpha 4\beta\gamma 2$ GABAR

In addition to increasing $\alpha 4\beta\delta$ GABAR expression, in some cases steroid withdrawal can also increase expression of $\alpha 4\beta\gamma 2$ GABAR subtypes (Follesa et al., 2001; Smith et al., 1998a), including pregnancy and pseudopregnancy in rats (Sanna et al., 2009; Smith et al., 1998b). GABARs containing $\gamma 2$ instead of δ exhibit a unique pharmacological profile (Wafford et al., 1996). Although both $\alpha 4\beta\delta$ and $\alpha 4\beta\gamma 2$ GABAR are insensitive to modulation by benzodiazepine agonists, current gated by $\alpha 4\beta\gamma 2$ GABAR is increased by the benzodiazepine antagonist flumazenil and the partial inverse agonist RO15-4513 (Wafford et al., 1996). In contrast, current gated by $\alpha 4\beta\delta$ GABAR is decreased by flumazenil (Dunn et al., 2003). Withdrawal from $3\alpha,5\alpha$ -THP following chronic administration of exogenous progesterone or $3\alpha,5[\alpha]\beta$ -THP to female rats increases $\alpha 4\beta\gamma 2$ GABAR, as evidenced by these characteristic changes in GABAR pharmacology, recorded from CA1 hippocampal pyramidal cells (Smith et al., 1998a).

Withdrawal from $3\alpha,5\alpha$ -THP could be produced by selective administration of a 5α -reductase blocker in the presence of high levels of progesterone (Smith et al., 1998b), confirming that $3\alpha,5\alpha$ -THP was the active component, even under conditions of high ovarian production in a pseudopregnancy model. Administration of other GABA modulatory drugs, such as benzodiazepines and ethanol, increase expression of $\alpha 4\beta\gamma 2$ GABAR, either after chronic administration or following withdrawal from the drug (Devaud et al., 1997; Follesa et al., 2002; Holt et al., 1996; Holt et al., 1997; Liang et al., 2004; Mahmoudi et al., 1997; Sanna et al., 2003), suggesting it may be an outcome of prolonged exposure to positive GABA-modulators, in general. It is not clear, however, why steroid withdrawal may lead to either increased expression of $\alpha 4\beta\delta$ or $\alpha 4\beta\gamma 2$, although the background steroid level may play a role.

Receptor kinetics

The functional consequence of increased expression of $\alpha 4\beta\gamma 2$ GABAR would be to result in synaptic receptors with a faster deactivation time constant (Smith and Gong, 2005), yielding less inhibitory current. In fact, recombinant $\alpha 4\beta 2\gamma 2$ GABAR deactivate more quickly than either $\alpha 1\beta 2\gamma 2$, $\alpha 3\beta 2\gamma 2$ or $\alpha 5\beta 2\gamma 2$ (Gingrich et al., 1995; Lavole et al., 1997; Smith and Gong, 2005), as do synaptic currents reflecting $\alpha 4\beta\gamma 2$ GABAR (Hsu et al., 2003; Cagetti et al., 2003; Chandra et al., 2006). Unlike tonic current, faster synaptic current would decrease feedback inhibition (Hsu and Smith, 2003), resulting in hyperexcitability and increasing pharmacologically induced seizure susceptibility (Smith et al., 1998a). This decrease in

feedback inhibition and increased seizure susceptibility was indeed observed after $3\alpha,5\alpha$ -THP withdrawal in female rats, an outcome reversed by suppression of $\alpha 4$ expression using intraventricular administration of antisense oligonucleotides (Hsu and Smith, 2003; Smith et al., 1998a).

Corticosterone, stress and puberty

In addition to $3\alpha,5[\alpha]\beta$ -THP, stress increases release of corticosteroids (Goldstein and Kopin, 2008), which constitute an important component of the stress reaction. Initially, activation of the HPA (hypothalamo-pituitary-adrenal) axis increases CNS release of CRH (corticotrophin releasing hormone), which has been shown to increase anxiety (Holsboer and Ising, 2008; Sahuque et al., 2006), although some reports suggest that this effect can be mediated by effects via the GABAergic system (Waselus et al., 2005). In addition, CRH acts to increase corticosterone release. Globally, corticosterone acts to increase circulating levels of glucose as fuel for the stress reaction (Goldstein and Kopin, 2008). However, corticosterone also affects hippocampal excitability, in both a concentration- and time-dependent manner, where fast effects are excitatory and longer-term effects are inhibitory (Birnstiel et al., 1995; Olijslagers et al., 2008; Rey et al., 1987). Effects of corticosterone depend on the area of hippocampus as well as the receptor subtype which is activated: in ventral hippocampus, activation of mineralocorticoid receptors decreases the frequency of sIPSCs, while activation of glucocorticoid receptors increases the amplitude of sIPSCs (Maggio and Segal, 2009). In dorsal hippocampus, however, corticosteroid acts only via the glucocorticoid receptors to increase sIPSC amplitude (Maggio and Segal, 2009). Corticosterone also affects both the amplitude and expression of L-type calcium channels in hippocampus (Chameau et al., 2007). In addition, long-term stress effects mediated via corticosterone produce genomic changes, such as alterations in kainate receptor mRNA in hippocampus (Hunter et al., 2009).

In addition to these complex effects of corticosterone mediated via selective receptor sub-types, corticosterone is also converted to THDOC, another positive modulator of the GABAR, which has been shown to be increased by stress (Barbaccia et al., 1996). Thus, the effects of this steroid are complex and involve both excitatory and inhibitory levels of control. Recent studies have demonstrated a greater corticosterone response to stress in pre-pubertal rats of both sexes than adults (Romeo et al., 2006; Romeo et al., 2004), suggesting that this hormonal response also changes at puberty.

Conclusions

In conclusion, the onset of puberty is associated with a striking upregulation of $\alpha 4\beta\delta$ GABARs along the apical dendrites of CA1 hippocampal pyramidal cells from almost undetectable levels before puberty. In areas with normally high expression of these receptors, dentate gyrus and cortical pyramidal cells, Cl⁻ flux is outward, where $3\alpha,5[\alpha]\beta$ -THP would increase inhibition. However, $\alpha 4\beta 2\delta$ GABAR are inhibited by $3\alpha,5[\alpha]\beta$ -THP under conditions of inward Cl⁻ flux, as would occur in mature hippocampus. Thus, this steroid decreases tonic inhibitory current at puberty, leading to increased neuronal excitability at the circuit level and increasing anxiety at the behavioral level. Because circulating levels of $3\alpha,5[\alpha]\beta$ -THP are increased by stress, these findings may be relevant for mood swings and the increased response to stress reported at the onset of puberty. Anxiety-producing effects of $3\alpha,5[\alpha]\beta$ -THP mediated by $\alpha 4\beta\delta$ GABAR may also be relevant for other hormonally-related changes in mood, such as PMDD, because they are triggered by fluctuating levels of endogenous neuroactive steroids.

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Reference List

- Agis-Balboa RC, Pinna G, Zhubi A, Maloku E, Veldic M, Costa E, Guidotti A. Characterization of brain neurons that express enzymes mediating neurosteroid biosynthesis. *Proc Natl Acad Sci* 2006;103:14602–14607. [PubMed: 16984997]
- Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behav Brain Res* 1999;106:119–125. [PubMed: 10595427]
- Andreen L, Sundstrom-Poromaa I, Bixo M, Andersson A, Nyberg S, Backstrom T. Relationship between allopregnanolone and negative mood in postmenopausal women taking sequential hormone replacement therapy with vaginal progesterone. *Psychoneuroendocrinology* 2004;30:212–224. [PubMed: 15471618]
- Apter D, Hermanson E. Update on female pubertal development. *Curr Opin Obstet Gynecol* 2002;14:475–481.
- Bai D, Zhu G, Pennefather P, Jackson MF, Macdonald JF, Orser BA. Distinct functional and pharmacological properties of tonic and quantal inhibitory postsynaptic currents mediated by γ -aminobutyric acid (A) receptors in hippocampal neurons. *Mol Pharm* 2000;59:814–824.
- Barbaccia ML, Concas A, Roscetti G, Bolacchi F, Mostallino MC, Purdy RH, Biggio G. Stress-induced increase in brain neuroactive steroids: Antagonist by abercamil. *Pharmacol Biochem Behav* 1996;54:205–210. [PubMed: 8728559]
- Barrera NP, Betts T, You H, Henderson RM, Martin IL, Dunn SM, Edwardson JM. Atomic force microscopy reveals the stoichiometry and subunit arrangement of the $\alpha 4\beta 3\delta$ GABA(A) receptor. *Mol Pharm* 2009;73:960–967.
- Belelli D, Casula A, Ling A, Lambert JJ. The influence of subunit composition on the interaction of neurosteroids with GABA (A) receptors. *Neuropharm* 2002;43:651–661.
- Belelli D, Herd MB. The contraceptive agent Provera enhances GABA_A receptor-mediated inhibitory neurotransmission in the rat hippocampus: evidence for endogenous neurosteroids? *J Neurosci* 2003;23:10013–10020. [PubMed: 14602815]
- Belelli D, Peden DR, Rosahl TW, Wafford K, Lambert JJ. Extrasynaptic GABA-A receptors for thalamocortical neurons: A molecular target for hypnotics. *J Neurosci* 2005;25:11513–11520. [PubMed: 16354909]
- Bianchi MT, Haas KF, Macdonald RL. Alpha1 and alpha6 subunits specify distinct desensitization, deactivation and neurosteroid modulation of GABA (A) receptors containing the delta subunit. *Neuropharm* 2002;43:492–502.
- Bianchi MT, Macdonald RL. Neurosteroids shift partial agonist activation of GABA (A) receptor channels from low- to high-efficacy gating patterns. *J Neurosci* 2003;23:10934–10943. [PubMed: 14645489]
- Birnsteil S, List TJ, Beck SG. Chronic corticosterone treatment maintains synaptic activity of CA1 hippocampal pyramidal cells: acute high corticosterone administration increases action potential number. *Synapse* 1995;20:117–124. [PubMed: 7570340]
- Bitran D, Dugan M, Renda P, Ellis R, Foley M. Anxiolytic effects of the neuroactive steroid pregnanolone (3alpha-OH-5beta-pregnan-20-one) after microinjection in the dorsal hippocampus and lateral septum. *Brain Res* 1999;850:217–224. [PubMed: 10629767]
- Bitran D, Hilvers RJ, Kellog CK. Anxiolytic effects of 3alpha-hydroxy-5alpha[β]-pregnan-20-one: Endogenous metabolites of progesterone that are active at the GABA_A receptor function. *Brain Res* 1993;561:157–161. [PubMed: 1686744]
- Brack KE, Lovick TA. Neuronal excitability in the periaqueductal grey matter during the estrous cycle in female Wistar rats. *Neuroscience*. 2006 epub Oct. 10.

- Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M. Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 2001;409:88–92. [PubMed: 11343119]
- Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human alpha (4)beta (3)delta GABA (A) receptors. *Br J Pharmacol* 2002;136:965–974. [PubMed: 12145096]
- Buchanan CM, Eccles JS, Becker JB. Are adolescents the victims of raging hormones: evidence for activational effects of hormones on moods and behavior at adolescence. *Psychol Bull* 1992;111:62–107. [PubMed: 1539089]
- Burgard EC, Tietz EI, Neelands TR, Macdonald RL. Properties of recombinant gamma-aminobutyric acid A receptor isoforms containing the alpha 5 subunit subtype. *Mol Pharmacol* 1996;50:119–127. [PubMed: 8700104]
- Cagetti E, Liang J, Spigelman I, Olsen RW. Withdrawal from chronic intermittent ethanol treatment changes subunit composition, reduces synaptic function, and decreases behavioral responses to positive allosteric modulators of GABA(A) receptors. *Mol Pharmacol* 2003;63:53–64. [PubMed: 12488536]
- Carascos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford K, MacDonald JF, Orser BA. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha 5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci* 2004;101:3662–3667. [PubMed: 14993607]
- Chameau P, Qin Y, Spijker S, Smit G, Joels M. Glucocorticoids specifically enhance L-type calcium current amplitude and affect calcium channel subunit expression in the mouse hippocampus. *J Neurophysiol* 2007;97:5–14. [PubMed: 17021021]
- Chandra D, Jia F, Liang J, Peng Z, Suryanarayanan A, Werner DF, Spigelman I, House CR, Olsen RW, Harrison NL, Homanics GE. GABA(A) receptor alpha-4 subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc Natl Acad Sci* 2006;103:15230–15235. [PubMed: 17005728]
- Chang Y, Wang R, Barot S, Weiss DS. Stoichiometry of a recombinant GABA-A receptor. *J Neurosci* 1990;16:534–541.
- Chen J, Mitcheson JS, Lin M, Sanguinetti MC. Functional roles of charged residues in the putative voltage sensor of the HCN2 pacemaker channel. *J Biol Chem* 2000;275:36465–36471. [PubMed: 10962006]
- Corpechot C, Collins B, Carey M, Tsouros A, Robel P, Fry J. Brain neurosteroids during the mouse oestrous cycle. *Brain Res* 1997;766:276–280. [PubMed: 9359616]
- Costello EJ, Sung M, Worthman C, Angold A. Pubertal maturation and the development of alcohol use and abuse. *Drug Alcohol Depend* 2007;88:S50–S59. [PubMed: 17275214]
- Devaud LL, Fritschy JM, Sieghar W, Morrow AL. Bidirectional alterations of GABA_A receptor subunit peptide levels in rat cortex during chronic ethanol consumption and withdrawal. *J Neurochem* 1997;69:126–130. [PubMed: 9202302]
- Drooglever Fortuyn HA, van Broekhoven F, Span PN, Backstrom T, Zitman FG, Verkes RJ. Effects of Ph.D. examination stress on allopregnanolone and cortisol plasma levels and peripheral benzodiazepine receptor density. *Psychoneuroendocrinology* 2004;29:1341–1344. [PubMed: 15288714]
- Dunn SMJ, Tancowny B, Martin IL. Characterisation of GABA-A receptors containing alpha4-beta1-delta subunits. *J Physiol* 2003;548, 5.
- Endicott J, Amsterdam J, Eriksson E, Frank E, Freeman E, Hirschfeld R, Ling F, Parry B, Pearlstein T, Rosenbaum J, Rubinow D, Schmidt P, Severino S, Steiner M, Stewaart D, Thys-Jacobs S. Is premenstrual dysphoric disorder a distinct clinical entity? *J Womens Health Gend Based Med* 1999;8:663–679. [PubMed: 10839653]
- Fadalti M, Petraglia F, Luisi S, Bernardi F, Casarosa E, Ferrari E, Luisi M, Saggese G, Genazzani AR, Bernasconi S. Changes of serum allopregnanolone levels in the first 2 years of life and during pubertal development. *Pediatric Res* 1999;46:323–327.
- Fancsik A, Linn DM, Tasker JG. Neurosteroid modulation of GABA IPSCs is phosphorylation dependent. *J Neurosci* 2000;20:3067–3075. [PubMed: 10777770]

- Feng HJ, Kang JQ, Song L, Dibbens L, Mulley J, Macdonald RL. Delta subunit susceptibility variants E177A and R220H associated with complex epilepsy alter channel gating and surface expression of $\alpha 4\beta 2\delta$ GABA-A receptors. *J Neurosci* 2006;26:1499–1506. [PubMed: 16452673]
- Follesa P, Cagetti E, Mancuso L, Biggio F, Manca A, Maciocco E, Massa F, Desole M, Carta M, Busonero F, Sanna E, Biggio G. Increase in expression of the GABA (A) receptor $\alpha 4$ subunit gene induced by withdrawal of, but not by long-term treatment with, benzodiazepine full or partial agonists. *Brain Res Mol Brain Res* 2001;92:138–148. [PubMed: 11483250]
- Follesa P, Mancuso L, Biggio F, Cagetti E, Franco M, Trapani G, Biggio G. Changes in GABA (A) receptor gene expression induced by withdrawal of, but not by long-term exposure to, zaleplon or zolpidem. *Neuropharm* 2002;42:191–198.
- Freeman EW, Frye CA, Rickels K, Martin PA, Smith SS. Allopregnanolone levels and symptom improvement in severe premenstrual syndrome. *J Clin Psychopharmacol* 2002;22:516–520. [PubMed: 12352277]
- Frye CA. The neurosteroid $3\alpha,5\alpha$ -THP has antiseizure and possible neuroprotective effects in an animal model of epilepsy. *Brain Res* 1995;696:113–120. [PubMed: 8574658]
- Frye CA, Paris JJ, Rhodes ME. Increasing $3\alpha,5\alpha$ -THP following inhibition of neurosteroid biosynthesis in the ventral tegmental area reinstates anti-anxiety, social and sexual behavior of naturally receptive rats. *Reproduction* 2009;137:119–128. [PubMed: 18818272]
- Gingrich KL, Roberts WA, Kass RS. Dependence of the GABAA receptor gating kinetics on the α subunit isoform: implications for structure-function relations and synaptic transmission. *J Physiol* 1995;489:529–543. [PubMed: 8847645]
- Girdler SS, Beth Mechlin M, Light KC, Morrow AL. Ethnic differences in allopregnanolone concentrations in women during rest and following mental stress. *Psychophysiology* 2006;43:331–336. [PubMed: 16916428]
- Glykys J, Peng Z, Chandra D, Homanics GE, Houser CR, Mody I. A new naturally occurring GABA-A receptor subunit partnership with high sensitivity to ethanol. *Nat Neurosci* 2007;10:40–48. [PubMed: 17159992]
- Glykys J, Mody I. The main source of ambient GABA responsible for tonic inhibition in the mouse hippocampus. *J Physiol* 2007;582:1163–1178. [PubMed: 17525114]
- Goldstein DS, Kopin JJ. Adrenomedullary, adrenocortical and sympathoneural responses to stressors: A meta-analysis. *Endocrine Regulations* 2008;42:111–119. [PubMed: 18999898]
- Griffiths J, Lovick T. Withdrawal from progesterone increases expression of $\alpha 4$, $\beta 1$ and δ GABA (A) receptor subunits in neurons in the periaqueductal gray matter in female Wistar rats. *J Comp Neurol* 2005;486:89–97. [PubMed: 15834956]
- Guo AL, Petraglia F, Criscuolo M, Ficarra G, Nappi RE, Palumbo MA, Trentini GP, Purdy RH, Gennazani AR. Evidence for a role of neurosteroids in modulation of diurnal changes and acute stress-induced corticosterone secretion in rats. *Gynecol Endocrinol* 1995;9:1–7. [PubMed: 7793294]
- Haas KF, Macdonald RL. GABA_A receptor subunit $\gamma 2$ and δ subtypes confer unique kinetic properties on recombinant GABA_A receptor currents in mouse fibroblasts. *J Physiol* 1999;514:27–45. [PubMed: 9831714]
- Halbreich U, Backstrom T, Eriksson E, O'Brien S, Calil H, Ceskova E, Dennerstein L, Douki S, Freeman EW, Genazzani AR, Heuser I, Kadri N, Rapkin A, Steiner M, Wittchen HU, Yonkers KA. Clinical diagnostic criteria for premenstrual syndrome and guidelines for their quantification for research studies. *Gynecol Endocrinol* 2007;23:123–130. [PubMed: 17454164]
- Hamann M, Rossi DJ, Attwell D. Tonic and spillover inhibition of granule cells control information flow through cerebellar cortex. *Neuron* 2002;33:625–633. [PubMed: 11856535]
- Harney SC, Frenguelli BG, Lambert JJ. Phosphorylation influences neurosteroid modulation of synaptic GABAA receptors in rat CA1 and dentate gyrus neurones. *Neuropharmacology* 2003;45:873–883. [PubMed: 14529725]
- Hayward C, Sanborn K. Puberty and the emergence of gender differences in psychopathology. *J Adolescent Health* 2002;30S:49–58.
- Herd MB, Haythornthwaite AR, Rosahl TW, Wafford K, Homanics GE, Lambert JJ, Belelli D. The expression of GABA-A β subunit isoforms in synaptic and extrasynaptic receptor populations of mouse dentate gyrus granule cells. *J Physiol* 2008;586:989–1004. [PubMed: 18079158]

- Hevers W, Luddens H. The diversity of GABA_A receptors. Pharmacological and electrophysiological properties of GABA_A channel subtypes. *Mol Neurobiol* 1998;18:35–86. [PubMed: 9824848]
- Higashi T, Takido N, Shimada K. Studies on neurosteroids XVII. Analysis of stress-induced changes in neurosteroid levels in rat brains using liquid chromatography-electron capture atmospheric pressure chemical ionization-mass spectrometry. *Steroids* 2005;70:1–11. [PubMed: 15610891]
- Holsboer F, Ising M. Central CRH system in depression and anxiety--evidence from clinical studies with CRH1 receptor antagonists. *Eur J Pharmacol* 2008;583:350–357. [PubMed: 18272149]
- Holt RA, Bateson AN, Martin IL. Chronic treatment with diazepam or abecarnil differently affects the expression of GABA_A receptor subunit mRNAs in the rat cortex. *Neuropharmacology* 1996;35:1457–1463. [PubMed: 9014161]
- Holt RA, Bateson AN, Martin IL. Chronic zolpidem treatment alters GABA (A) receptor mRNA levels in the rat cortex. *Eur J Pharmacol* 1997;329:129–132.
- Hosie AM, Wilkins ME, da Silva HM, Smart TG. Endogenous neurosteroids regulate GABA-A receptors through two discrete transmembrane sites. *Nature* 2006;444:486–489. [PubMed: 17108970]
- Hsu FC, Smith SS. Progesterone withdrawal reduces paired-pulse inhibition in rat hippocampus: Dependence on GABA-A receptor alpha-4 upregulation. *J Neurophysiol* 2003;89:186–198. [PubMed: 12522171]
- Hsu FC, Waldeck R, Faber DS, Smith SS. Neurosteroid effects on GABAergic synaptic plasticity in hippocampus. *J Neurophysiol* 2003;89:1929–1940. [PubMed: 12612040]
- Huerta R, Brizuela-Gamino OL. Interaction of pubertal status, mood and self-esteem in adolescent girls. *J Reprod Med* 2002;47:217–225. [PubMed: 11933687]
- Hunter RG, Bellani R, Bloss E, Costa A, McCarthy K, McEwen BS. Regulation of kainate receptor subunit mRNA by stress and corticosteroids in the rat hippocampus. *PLoS ONE* 2009;4:e4328. [PubMed: 19180187]
- Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA. An extrasynaptic GABA-A receptor mediates tonic inhibition in thalamic VB neurons. *J Neurophysiol* 2005;94:4491–4501. [PubMed: 16162835]
- Kahn A, Berger RG, deCatanzaro D. The onset of puberty in female mice as reflected in urinary steroids and uterine/ovarian mass: interactions of exposure to males, phytoestrogen content of diet, and anogenital distance. *Reproduction* 2008;235:99–106.
- Kask K, Backstrom T, Lundgren P, Sundstrom-Poromaa I. Allopregnanolone has no effect on startle response and prepulse inhibition of startle response in patients with premenstrual dysphoric disorder or healthy controls. *Pharmacol Biochem Behav*. 2009 epub March 4, 2009.
- Kelley SP, Dunlop JJ, Kirkness E, Lambert JJ, Peters JA. A cytoplasmic region determines single-channel conductance in 5-HT₃ receptors. *Nature* 2003;424:321–324. [PubMed: 12867984]
- Lancel M, Faulhaber J, Schiffelholz T, Romeo E, Di Michele F, Holsboer F, Rupprecht R. Allopregnanolone affects sleep in a benzodiazepine-like fashion. *J Pharmacol Exp Ther* 1997;282:1213–1218. [PubMed: 9316828]
- Lavole AM, Tingey JJ, Harrison NL, Pritchett DB, Twyman RE. Activation and Deactivation Rates of Recombinant GABA_A Receptor Channels Are Dependent on α -Subunit Isoform. *Biophysical Journal* 1997;73:1–9. [PubMed: 19431906]
- Le Melleo JM, Van Driel M, Coupland NJ, Lott P, Jhangri GS. Response to flumazenil in women with premenstrual dysphoric disorder. *Am J Psychiatry* 2000;157:821–823. [PubMed: 10784479]
- Liang J, Cagetti E, Olsen RW, Spigelman I. Altered Pharmacology of Synaptic and Extrasynaptic GABA-A Receptors on CA1 Hippocampal Neurons Is Consistent with Subunit Changes in a Model of Alcohol Withdrawal and Dependence. *J Pharmacol Exp Ther* 2004;310:1234–1245. [PubMed: 15126642]
- Liang J, Suryanarayanan A, Chandra D, Homanics GE, Olsen RW, Spigelman I. Functional consequences of GABA_A receptor alpha 4 subunit deletion on synaptic and extrasynaptic currents in mouse dentate granule cells. *Alcohol Clin Exp Res* 2008;32:19–26. [PubMed: 18070250]
- Lo M, Snyder SH. Two distinct solubilized benzodiazepine receptors: differential modulation by ions. *J Neurosci* 1983;3:2270–2279. [PubMed: 6313875]
- Lovick T. Panic disorder: a malfunction of multiple transmitter control systems within the midbrain periaqueductal gray matter? *Neuroscientist* 2000;6:48–59.

- Lovick TA, Griffiths JL, Dunn SM, Martin IL. Changes in GABA (A) receptor subunit expression in the midbrain during the oestrous cycle in Wistar rats. *Neuroscience* 2005;131:397–405. [PubMed: 15708482]
- Lundgren P, Stromberg J, Backstrom T, Wang M. Allopregnanolone-stimulated GABA-mediated chloride ion flux is inhibited by 3beta-hydroxy-5alpha-pregnan-20-one (isoallopregnanolone). *Brain Res* 2003;982:45–53. [PubMed: 12915239]
- Maggio N, Segal M. Differential corticosteroid modulation of inhibitory synaptic currents in the dorsal and ventral hippocampus. *J Neurosci* 2009;29:2857–2866. [PubMed: 19261881]
- Maguire JL, Mody I. Neurosteroid synthesis-mediated regulation of GABA (A) receptors: relevance to the ovarian cycle and stress. *J Neurosci* 2007;27:2155–2162. [PubMed: 17329412]
- Maguire JL, Mody I. GABA(A)R plasticity during pregnancy: relevance to postpartum depression. *Neuron* 2009;59:207–213. [PubMed: 18667149]
- Maguire JL, Stell BM, Rafizadeh M, Mody I. Ovarian cycle-linked changes in GABA (A) receptors mediating tonic inhibition alter seizure susceptibility and anxiety. *Nat Neurosci* 2005;8:797–804. [PubMed: 15895085]
- Mahmoudi M, Kang MH, Tillakaratne N, Tobin AJ, Olsen RW. Chronic intermittent ethanol treatment in rats increases GABA_A receptor $\alpha 4$ subunit expression - possible relevance to alcohol dependence. *J NeuroChem* 1997;68:2485–2492. [PubMed: 9166743]
- Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* 1986;232:1004–1007. [PubMed: 2422758]
- McCartney CR, et al. Obesity and sex steroid changes across puberty: Evidence for marked hyperandrogenemia in pre- and early pubertal obese girls. *J Clin Endocrinol Metab* 2007;92:430–436. [PubMed: 17118995]
- McEwen BS. Stressed or stressed out: what is the difference? *J Psychiatr Neurosci* 2005;30:315–318.
- Mellon SH, Vaudry H. Biosynthesis of neurosteroids and regulation of their synthesis. *Int Rev Neurobiol* 2001;46:33–78. [PubMed: 11599305]
- Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li ZW, DeLorey TM, Olsen RW, Homanics GE. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyric acid type A receptor delta subunit knockout mice. *Proc Natl Acad Sci* 1999;96:12905–12910. [PubMed: 10536021]
- Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* 2003;424:949–955. [PubMed: 12827192]
- Modesti PA, Pela I, Cecioni I, Gensini GF, Sernerri GG, Bartolozzi G. Changes in blood pressure reactivity and 24-hour blood pressure profile occurring at puberty. *Angiology* 2006;45:443–450. [PubMed: 8203770]
- Mitchellishvili Z, Kapur J. High-affinity, slowly desensitizing GABA-A receptors mediate tonic inhibition in hippocampal dentate granule cells. *Mol Pharmacol* 2006;69:564–575. [PubMed: 16282519]
- Mukai H, Higashi T, Nagura Y, Shimada K. Studies on neurosteroids XXV. Influence of 5alpha-reductase inhibitor, finasteride, on rat brain neurosteroid levels and metabolism. *Biol Pharm Bull* 2008;31:1646–1650. [PubMed: 18758053]
- Olijslagers JE, de Kloet ER, Elgersma Y, van Woerden GM, Joels M, Karst H. Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors. *Eur J Neuroscience* 2008;27:2542–2550.
- Olsen RW, Sieghart W. International Union of Pharmacology. LXX Subtypes of gamma-aminobutyric acid (A) receptors: classification on the basis of subunit composition, pharmacology and function. *Pharmacol Rev* 2008;60:243–260. [PubMed: 18790874]
- Olsen RW, Snowman A. Chloride-dependent enhancement by barbiturates of gamma-aminobutyric acid receptor binding. *J Neuroscience* 1982;2:1812–1823.
- Palumbo MA, Salvestroni C, Gallo R, Guo AL, Gennazini AD, Artini PG, Petraglia F, Gennazani AR. Allopregnanolone concentration in hippocampus of prepubertal rats and female rats throughout estrous cycle. *J Endo Invest* 1995;18:853–856.

- Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J Neurosci Methods* 1995;14:149–167. [PubMed: 2864480]
- Perkins KL. Cell-attached voltage-clamp and current-clamp recording and stimulation techniques in brain slices. *J Neurosci Methods* 2006;154:1–18. [PubMed: 16554092]
- Porcello DM, Huntsman MM, Mihalek RM, Homanics GE, Huguenard JR. Intact synaptic GABAergic inhibition and altered neurosteroid modulation of thalamic relay neurons in mice lacking δ subunit. *J Neurophysiol* 2003;89:1378–1386. [PubMed: 12626617]
- Purdy RH, Morrow AL, Moore PH Jr, Paul SM. Stress-induced elevations of gamma-aminobutyric acid type A receptor-active steroids in the rat. *PNAS* 1991;88:4553–4557. [PubMed: 1852011]
- Ramsey IS, Moran MM, Chong JA, Clapham DE. A voltage-gated proton-selective channel lacking the pore domain. *Nature* 2006;440:1213–1216. [PubMed: 16554753]
- Rey M, Carlier E, Soumireu-Mourat B. Effects of corticosterone on hippocampal slice electrophysiology in normal and adrenalectomized BALB/c mice. *Neuroendocrinology* 1987;46:424–429. [PubMed: 3431658]
- Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Saarma M, Kaila K. The K-Cl cotransporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 1999;397:251–255. [PubMed: 9930699]
- Romeo RD, Karatsoreos IN, McEwen BS. Pubertal maturation and time of day differentially affect behavioral and neuroendocrine responses following an acute stressor. *Horm Behav* 2006;50:463–8. [PubMed: 16836997]
- Romeo RD, Lee SJ, McEwen BS. Differential stress reactivity in intact and ovariectomized prepubertal and adult female rats. *Neuroendocrinology* 2004;80:387–393. [PubMed: 15741744]
- Rudolph U, Crestani F, Benke D, Brünig I, Benson JA, Fritschy JM, Martin JR, Bluethmann H, Möhler H. Benzodiazepine actions mediated by specific γ -aminobutyric acid_A receptor subtypes. *Nature* 1999;401:796–800. [PubMed: 10548105]
- Safranski TJ, Lamberson WR, Keisler DH. Correlations among three measures of puberty in mice and relationships with estradiol concentration and ovulation. *Biology of Reproduction* 1993;48:669–673. [PubMed: 8452942]
- Sahuque L, Kullberg EF, McGeehan AJ, Kinder JR, Hicks MP, Blanton MG, Janak PH, Olive MF. Anxiogenic and aversive effects of corticotrophin releasing factor (CRF) in the bed nucleus of the stria terminalis of the rat: Role of CRF receptor subtypes. *Psychopharm* 2006;186:122–132.
- Sanna E, Mostallino MC, Busonero F, Talani G, Tranquilli S, Mameli M, Spiga R, Follesa P, Biggio G. Changes in GABA-A receptor gene expression associated with selective alterations in receptor function and pharmacology after ethanol withdrawal. *J Neurosci* 2003;23:11711–11724. [PubMed: 14684873]
- Sanna E, Mostallino MC, Murru L, Carta M, Talani G, Zucca S, Mura ML, Maciocco E, Biggio G. Changes in expression and function of extrasynaptic GABA-A receptors in the rat hippocampus during pregnancy and after delivery. *J Neurosci* 2009;29:1755–1765. [PubMed: 19211882]
- Saxena NC, Neelands TR, Macdonald RL. Contrasting actions of lanthanum on different recombinant gamma-aminobutyric acid receptor isoforms expressed in L929 fibroblasts. *Mol Pharmacol* 1997;51:328–335. [PubMed: 9203639]
- Schmidt P, Nieman L, Danaceau M, Adams L, Rubinow D. Differential behavioral effects of gonadal steroids in women with premenstrual syndrome. *New England J Med* 1998;338:209–216. [PubMed: 9435325]
- Scimemi A, Andersson A, Heeroma JH, Strandberg J, Rydenhag B, McEvoy AW, Thom M, Asztely F, Walker MC. Tonic GABAA receptor mediated currents in human brain. *Eur J Neurosci* 2006;24:1157–1160. [PubMed: 16930441]
- Shen H, Gong QH, Aoki C, Yuan M, Ruderman Y, Dattilo M, Williams K, Smith SS. Reversal of neurosteroid effects at $\alpha 4\beta 2\delta$ GABA-A receptors triggers anxiety at puberty. *Nat Neurosci* 2007;10:469–477. [PubMed: 17351635]
- Shen H, Gong QH, Yuan M, Smith SS. Short-term steroid treatment increases δ GABA-A receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. *Neuropharmacology* 2005;49:573–586. [PubMed: 15950994]

- Smith SS, Gong QH. Neurosteroid administration and withdrawal alter GABA-A receptor kinetics in CA1 hippocampus of female rats. *J Physiol* 2005;564:421–436. [PubMed: 15705652]
- Smith SS, Gong QH, Hsu FC, Markowitz RS, French-Mullen JMH, Li X. GABA_A receptor $\alpha 4$ subunit suppression prevents withdrawal properties of an endogenous steroid. *Nature* 1998a;392:926–929. [PubMed: 9582073]
- Smith SS, Gong QH, Li X, Moran MH, Bitran D, Frye CA, Hsu FC. Withdrawal from 3 α -OH-5 α -pregnan-20-one withdrawal using a pseudopregnancy model alters the kinetics of hippocampal GABA_A-gated current and increases the GABA_A receptor $\alpha 4$ subunit in association with increased anxiety. *J of Neuroscience* 1998b;18:5275–5284.
- Smith SS, Ruderman Y, Frye CA, Homanics GE, Yuan M. Steroid withdrawal in the mouse results in anxiogenic effects of 3 α ,5 α -THP: A possible model of premenstrual dysphoric disorder. *Psychopharmacology* 2006;186:323–333. [PubMed: 16193334]
- Spigelman I, Li ZW, Liang J, Cagetti E, Samzadeh S, Mihalek RM, Homanics GE, Olsen RW. Reduced inhibition and sensitivity to neurosteroids in hippocampus of mice lacking the GABA(A) receptor delta subunit. *J Neurophysiol* 2003;90:903–910. [PubMed: 12702713]
- Stell BM, Brickley SG, Tang CY, Farrant M, Mody I. Neuroactive steroids reduce neuronal excitability by selectively enhancing tonic inhibition mediated by delta subunit-containing GABA-A receptors. *Proc Natl Acad Sci* 2003;100:14439–14444. [PubMed: 14623958]
- Stell BM, Mody I. Receptors with different affinities mediate phasic and tonic GABA (A) conductances in hippocampal neurons. *J Neurosci* 2002;22:RC223. [PubMed: 12006605]
- Sundstrom-Poromaa I, Smith DH, Gong Q, Sabado TN, Li X, Light A, Wiedmann M, Williams K, Smith S. Hormonally regulated $\alpha 4\beta 2\delta$ GABA-A receptors are a target for alcohol. *Nat Neurosci* 2002;5:721–722. [PubMed: 12118257]
- Sundstrom I, Nyberg S, Backstrom T. Patients with premenstrual syndrome have reduced sensitivity to midazolam compared to control subjects. *Neuropsychopharm* 1997;17:370–381.
- Turner JH, Raymond JR. Interaction of calmodulin with the serotonin 5-hydroxytryptamine_{2A} receptor. A putative regulator of G protein coupling and receptor phosphorylation by protein kinase C. *J Biol Chem* 2005;280:30741–30750. [PubMed: 15970592]
- van Broekhoven F, Backstrom T, Verkes RJ. Oral progesterone decreases saccadic eye velocity and increases sedation in women. *Psychoneuroendocrinology* 2006;31:1190–1199. [PubMed: 17034954]
- Voituron N, Frugiere A, Gros F, Macron JM, Bodineau L. Diencephalic and mesencephalic influences on ponto-medullary respiratory control in normoxic and hypoxic conditions: an in vitro study on central nervous system preparations from newborn rat. *Neuroscience* 2005;132:843–854. [PubMed: 15837144]
- Wafford KA, Thompson SA, Sikela J, Wilcox AS, Whiting PJ. Functional characterization of human GABA receptors containing the $\alpha 4$ subunit. *Mol Pharmacol* 1996;50:670–678. [PubMed: 8794909]
- Wallner M, Hancher HJ, Olsen RW. Ethanol enhances $\alpha 4\beta 2\delta$ and $\alpha 6\beta 3\delta$ GABA-A receptors at low concentrations known to affect humans. *Proc Nat Acad Sci* 2003;100:15218–15223. [PubMed: 14625373]
- Waselus M, Valentino RJ, Van Bockstaele EJ. Ultrastructural evidence for a role of gamma-aminobutyric acid in mediating the effects of corticotropin-releasing factor on the rat dorsal raphe serotonin system. *J Comp Neurol* 2005;482:155–165. [PubMed: 15611993]
- Wei W, Faria LC, Mody I. Low ethanol concentrations selectively augment the tonic inhibition mediated by δ subunit-containing GABA-A receptor in hippocampal neurons. *J Neurosci* 2004;24:8379–8382. [PubMed: 15385620]
- Wei W, Zhang N, Peng Z, Houser CR, Mody I. Perisynaptic localization of delta subunit-containing GABA (A) receptors and their activation by GABA spillover in the mouse dentate gyrus. *J Neurosci* 2003;23:10650–10661. [PubMed: 14627650]
- Wisden W, Laurie DJ, Monyer H, Seeburg P. The distribution of 13 GABA-A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 1992;12:1040–1062. [PubMed: 1312131]
- Wohlfarth KM, Bianchi MT, Macdonald RL. Enhanced neurosteroid potentiation of ternary GABA (A) receptors containing the delta subunit. *J Neurosci* 2002;22:1541–1549. [PubMed: 11880484]

- Wu Y, Wang W, Richerson G. GABA transaminase inhibition induces spontaneous and enhances depolarization-evoked GABA efflux via reversal of the GABA transporter. *J Neurosci* 2001;21:2630–2639. [PubMed: 11306616]
- Yonkers KA. Anxiety symptoms and anxiety disorders: how are they related to premenstrual disorders? *J Clin Psychiatry* 1997;58:62–69. [PubMed: 9133494]
- Zheleznova N, Sedelnikova A, Weiss DS. Alpha1-beta2-delta, a silent GABA-A receptor: recruitment by trazolate and neurosteroids. *Brit J Pharmacol* 2008;153:1062–1071. [PubMed: 18204487]

Figure 1.

The neurosteroid 3α,5[α]β-THP decreases inward Cl⁻ flux at α4β2δ GABA_A receptors. **(a)** Representative traces showing the effects of 30 nM 3α,5[α]β-THP (right) on current gated by 1 μM GABA (EC₇₅), under conditions of inward Cl⁻ flux (upper trace) and outward Cl⁻ flux (lower trace) for two δ-containing recombinant GABA_A receptor subtypes. The direction of Cl⁻ flux was reversed by varying internal Cl⁻ (upper trace, ECl⁻ = -70; lower trace, ECl⁻ = -30 mV), but using a constant holding potential of -50 mV. **(b)** Mean effects of 3α,5[α]β-THP on outward and inward Cl⁻ flux in response to 1 μM GABA (from 6 – 7 cells for each group; *P < 0.05 vs. the other receptor subtypes) **(c)** 3α,5[α]β-THP effects on desensitization of inward

(upper trace) and outward (lower trace) Cl^- flux through $\alpha 4\beta 2\delta$ receptors. This effect is representative of 6 cells for each group.

Intracellular loop of $\alpha 1$ and $\alpha 4$

a

$\alpha 1$

NYFTKRGYAWDGKSVVPEKPKVKDPLIKNNNTYAPTATSYTPNLNLRGDPGLATIAKSAT
NYFTNIQMEKAKRKTSKPPQEVPAAPVQREKHPEAPLQNTNANLNM~~MRKR~~TNALVHSESDV
NYFTNIQMOKAKKKISKPPPEVPAAPVLKEKHTETSLQNTNANLNM~~MRKR~~TNALVHSESDV

H α 4

IEPKEVKPETK-----
GNRTEVGNHSSKSSSTVVQESSKGTPRSYLASSPNPFSRANAETISAARALPSASPTSIR
KSRTTEVGNHSSKTS-AVQESSEATPKAHLASSPNPFSRANAETMSAAARGLSSAASPS
**

M α 4

-----PEPKKTFNSVSKIDR
TGYMPRKASVGSASTRHVFGSRLQRIKTTVNTIGATGKLSATPPPSAPPPSGSGTSKIDK
HGTLR-PASLGSASTRPAFGSRLGRIKTTVNTTGAAGNVSATPPPPAPPPSGSGTSKIDK
* * *****

b

Figure 2.

Arginine 353 in the $\alpha 4$ subunit is necessary for the direction-sensitive inhibition of $\alpha 4\beta 2\delta$ GABA_A receptors by $3\alpha, 5[\alpha]\beta$ -THP. **(a)** Alignment of the intracellular loop of $\alpha 1$ and $\alpha 4$ (H, human; M, mouse) subunits reveals limited identity (< 10%). *identical residues for all three. (The sequences for human and mouse $\alpha 1$ are identical.) Orange, residues to be mutated. **(b)** Representative traces showing the effect of 30 nM $3\alpha, 5[\alpha]\beta$ -THP on GABA(10 μ M)-gated current at the indicated mutated $\alpha 4\beta 2\delta$ GABA_A receptors. Basic arginine (R351 or R353) residues in the $\alpha 4$ subunit were mutated to a neutral glutamine (Q) and/or a basic lysine (K).

Figure 3.

$3\alpha,5[\alpha]\beta$ -THP lowers the current threshold for spiking of pyramidal cells at the onset of puberty. **(a)** Whole cell current clamp recordings conducted from CA1 hippocampal pyramidal cells. Voltage responses recorded in response to increasing 0.3 nA current injection (-1 nA, initial current) for slices recorded before puberty (Pre-pub), or at puberty in wild-type (Pub) or $\delta^{-/-}$ (Pub. $\delta^{-/-}$) mice. (The $3\alpha,5[\alpha]\beta$ -THP trace lacks the 800 pA current trace for ease of comparison.) Inset, spiking at threshold, 800 pA, pre- $3\alpha,5[\alpha]\beta$ -THP; 500 nA $3\alpha,5[\alpha]\beta$ -THP in a non-spiking pubertal cell. Red trace, equivalent current injection, threshold for the less excitable state. Blue trace, equivalent current injection, threshold for the more excitable state.) **(b)** Mean $\pm$ SEM averaged from 7 – 8 cells for each group. Current threshold to spiking, I

threshold; voltage threshold to spiking, *V_m threshold*; spike frequency, *No. of spikes*; action potential amplitude, *AP amplitude*; action potential half-width, *AP half-width*. Spike frequency was assessed at the minimum current required to produce spiking in both pre- and post-3 α ,5 [17]-TTP traces. *P < 0.05 versus Pre-pub.