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The influence of stress at puberty on mood and learning: Role of the α4βδ $GABA_A$ receptor

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Abstract

It is well-known that the onset of puberty is associated with changes in mood as well as cognition. Stress can have an impact on these outcomes, which in many cases, can be more influential in females, suggesting that gender differences exist. The adolescent period is a vulnerable time for the onset of certain psychopathologies, including anxiety disorders, depression and eating disorders, which are also more prevalent in females. One factor which may contribute to stress-triggered anxiety at puberty is the $GABA_A$ receptor (GABAR), which is known to play a pivotal role in anxiety. Expression of α4βδ GABARs increases on the dendrites of CA1 pyramidal cells at the onset of puberty in the hippocampus, part of the limbic circuitry which governs emotion. This receptor is a sensitive target for the stress steroid THP (3α-OH-5[α]β-pregnan-20-one), which paradoxically reduces inhibition and increases anxiety during the pubertal period (~PND 35–44) of female mice in contrast to its usual effect to enhance inhibition and reduce anxiety. Spatial learning and synaptic plasticity are also adversely impacted at puberty, likely a result of increased expression of α4βδ GABARs on the dendritic spines of CA1 hippocampal pyramidal cells, which are essential for consolidation of memory. This review will focus on the role of these receptors in mediating behavioral changes at puberty. Stress-mediated changes in mood and cognition in early adolescence may have relevance for the expression of psychopathologies in adulthood.

Keywords

Puberty; GABA-A receptor; alpha4; delta; anxiety; cognition

1.1 Introduction

It is well known that adolescence is a period frequently associated with behavioral and cognitive changes in humans, which can include mood swings (Buchanan et al., 1992), increased/altered response to stress (Modesti et al., 1994; Susman et al., 1988), risk-seeking behavior (Liang et al., 1995), altered social interaction, and, in some cases, decreases in CNS plasticity and cognition (Johnson and Newport, 1989; McGivern et al., 2002). It is also recognized as a particularly vulnerable time for the onset of certain psychiatric disorders, including generalized anxiety disorder (Reardon et al., 2009) and depression (Heim et al., 2004), which are more prevalent in females (Dorn and Chrousos, 1997), as well as a period

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when stress can influence the eventual development of some psychiatric disorders, such as schizophrenia, that occur later in adulthood (Corcoran et al., 2012; Dahl, 2004). Some studies have noted developmental milestones associated with chronological age which impact upon some of these cognitive and behavioral outcomes (Vetter-O'Hagen and Spear, 2012). However, the onset of puberty is associated with an array of hormonal events which converge to produce viable reproductive function. These same hormonal events may also contribute to some of the psychological and cognitive changes associated with puberty which are not directly linked to reproductive function.

Stress is defined as the body's reaction to environmental demands which elicit physical, mental or emotional responses, in most cases correlated with changes in heart rate and/or blood pressure. Most studies typically use performance stress (oral presentations, mental arithmetic, mirror tracing, etc.) for human stress evaluations and restraint stress or CO_2 inhalation for rodent studies. Stress steroids are steroids released as part of the reaction to stress. The most well-known stress hormones include epinephrine, norepinephrine and corticosterone; Animal studies have shown that corticosterone, in particular, produces profound effects on mood and cognition via acute and chronic effects on neuronal function and viability (Davidson and McEwen, 2012; McEwen, 2007; McEwen et al., 2012). However, this article will focus on the role of THP ([allo]pregnanolone, 3α-OH-5[α]β-pregnan-20-one) in mediating some of the changes in mood and cognition observed during the pubertal period. THP is also a steroid released by stress (Girdler et al., 2006; Higashi et al., 2005; Purdy et al., 1991), by the adrenal and CNS in both humans and rodents, which functions to modulate inhibition via direct effects on the $GABA_A$ receptor (GABAR), a receptor shown to play a pivotal role in anxiety (Rudolph et al., 1999; Trincavelli et al., 2012) in mice and humans.

1.2 Anxiety and adolescence

One of the most prevalent types of psychopathologies in human adolescents is anxiety (Costello et al., 2003). In fact, anxiety disorders with a lifetime course are most likely to begin at the time of puberty. In one study the median age of onset was 11 years (Kessler et al., 2005), consistent with pubertal onset, with more than 50% having an onset by age 14, during the pubertal period. There are also gender differences in psychopathology during adolescence, with girls more likely to develop an anxiety disorder (Hayward and Sanborn, 2002), for whom the most common subtypes reported are social anxiety and panic disorder (Costello et al., 2003; Zgourides and Warren, 1988). Both the prevalence and intensity of panic attacks have been correlated with pubertal status in girls (Hayward et al., 1992; Leen-Feldner et al., 2007).

1.3 Stress and adolescence

Response to a stressful experience is increased at the onset of puberty (Modesti et al., 2006; Sumter et al., 2010; Susman et al., 1988) in humans, where performance on a video game was shown to provoke increases in blood pressure, and the anticipatory stress of public speaking increased cortisol responses in adolescents to a greater degree than in younger children. Puberty is also widely regarded as the onset of gender differences in negative affect as a response to stress (Dorn and Chrousos, 1997; Ordaz and Luna, 2012). Many studies in humans have reported greater negative mood in females in early adolescence as a response to performance or psychosocial stressors (Garber et al., 2002; Ge et al., 2010), which is a trait that can extend into adulthood (Kelly et al., 2008). In studies investigating the autonomic changes triggered by stressors (mental arithmetic, mirror tracing, interpersonal stress) in early to mid-adolescence, females responded with a greater increase in heart rate compared to their male adolescent counterparts (Ewart and Kolodner, 1991;

Matthews et al., 1990; Syme et al., 2009). Anxiety responses to acute stressful stimuli are also correlated with the more advanced pubertal Tanner stages in females (Huerta and Brizuela-Gamino, 2002; Leen-Feldner et al., 2007). In addition to the response to acute stress, the response to chronic stress is exacerbated in adolescence: in individuals experiencing post-traumatic stress disorder, puberty onset was associated with an increase in negative affect, re-experience and hyperarousal (Carrion et al., 2002). Stress during adolescence can lead to different coping mechanisms, which may either be palliative or result in dysfunctional behavior, including risk-taking behavior and substance abuse.

Regional activity is increased in an age and gender-specific fashion throughout the cortico-limbic circuit which is involved in the stress response during adolescence, which includes the amygdala, hippocampus, insula and hypothalamus. Functional magnetic resonance imaging (fMRI) has been used to assess such activity during a psychosocial stress paradigm (Guyer et al., 2009). In females, increased activity of hypothalamus, insula, nucleus accumbens and hippocampus was observed during the stressor, an effect correlated with age. In contrast, for males, the stressor was associated with decreased insula activity, but no change in any of the other structures. Surprisingly, there was no change in amygdala activity during the task.

There are also gender differences in the size of these structures which are correlated with the onset of puberty, when amygdala volumes are greater in males, while hippocampal volumes are greater in females (Blanton et al., 2012; Lenroot and Giedd, 2010; Neufang et al., 2009), effects tightly correlated to pubertal status as well as to circulating levels of testosterone and estradiol, respectively. Intriguingly, sex differences in the growth of hippocampal neurons has been linked to circulating levels of E2 in males (Bowers et al., 2010). Taken together, these findings suggest that adolescence is a period when stress produces dynamic changes in both the structural and functional aspects of associated CNS circuits.

1.4 Puberty and pubertal hormones

Pubertal development in the human is a process which takes years to complete and can be defined by various end-points which culminate in reproductive function. The onset of reproductive function (gonadarche) can be identified in the human by functional markers which occur around 11–13 years of age, onset of spermatogenesis in the male (spermarche) and onset of menstruation in the female (menarche), which precede the first ovulation that usually occurs up to 1–3 years later (Apter and Hermanson, 2002; Tinggaard et al., 2012). Puberty can also be defined by outward physical signs, the secondary sexual characteristics, which are divided into Tanner Stages. Tanner stage 1 is pre-pubertal, and pubertal development proceeds across stages 2 – 5 (Apter and Hermanson, 2002; Tinggaard et al., 2012). In rodents, puberty onset is defined by pre-putial separation in the male and vaginal opening in the female, which occur on average at ~5 weeks of age (Tena-Sempere, 2010). In both humans and rodents, these external markers of puberty are preceded during the peri-pubertal period by increases in circulating levels of testosterone (T) or 17β-estradiol (E2), respectively (Apter and Hermanson, 2002; Janfaza et al., 2006; Safranski et al., 1993; Tena-Sempere, 2010; Tinggaard et al., 2012). In the female mouse, circulating levels of E2 increase more than two-fold five days before vaginal opening (Ahima et al., 1997). However, ovarian hormonal fluctuations are not evidenced until 10 d – 2 weeks after puberty onset (Hodes and Shors, 2005). In both humans and rodents, the age of puberty onset is variable, and influenced by a variety of factors including seasonal changes, body fat, nutrition, pheromones, etc. (Apter, 2003).

The onset of puberty in the human is preceded by maturation of the adrenal gland (adrenarche), which releases adrenal steroids, including the adrenal androgens

dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS), as well as progesterone (P), without releasing cortisol (Miller, 2009). Although adrenarche may not occur in the rodent, several studies have documented rising levels of P which precede puberty onset (Khan et al., 2008; Mannan and O'Shaughnessy, 1988). Thus, the circulating levels of multiple steroids change as puberty approaches. Many of these steroids have documented effects on mood and cognition in adult rodents (E2, T, P, DHEA, DHEAS) (Frye et al., 2007; Frye and Walf, 2009a; Frye and Walf, 2009b; Maninger et al., 2009; Reddy et al., 1998; Walf and Frye, 2008), suggesting that deciphering underlying mood changes at puberty may be a complex process. This review will focus on one steroid, THP ([allo]pregnanolone or 3α-OH-5[α]β-pregnan-20-one), a metabolite of P and a potent modulator of the $GABA_A$ receptor (GABAR), which has been shown to play a pivotal role in anxiety (Shen et al., 2007). For the purposes of this review, the onset of puberty in the female mouse will be defined by vaginal opening, and the prior 5 peri-pubertal days will be defined as “pre-pubertal”.

1.4.1 THP

THP is formed from P via two enzymatic conversions, with 5α-reductase and 3α-hydroxysteroid oxidoreductase (3α-HSD) (Compagnone and Mellon, 2000; Karavolas and Hodges, 1990; Mellon and Vaudry, 2001). This steroid can be produced in the adrenal gland, with release into the general circulation before puberty onset. It can also be converted from P produced by the granulosa cells of the ovary before puberty. In non-pregnant adult mice, P is also primarily produced by the granulosa cells on the afternoon of proestrus with lower levels produced by the corpus luteum on the diestrous1 stage of the estrous cycle (Bentley, 1998). (In contrast to humans, for whom the corpus luteum is the primary producer of this steroid.) In addition, recent studies suggest that THP can be produced *de novo* directly in the brain from cholesterol via side chain cleavage enzyme; thus, it is classified as a neurosteroid (Compagnone and Mellon, 2000). This can occur in a variety of CNS sites, in both glia and neurons, including the CA1 hippocampal pyramidal cell (Agis-Balboa et al., 2006). It is not clear, however, if these elevated pre-pubertal THP levels produce any effect on behavior or mood. Recent studies suggest that circulating and/or CNS levels of this steroid increase in the pre-pubertal period, but decline to low levels at the onset of puberty, when levels fluctuate on a circadian cycle (Fadalti et al., 1999; McCartney and et al., 2007). Unlike most steroids, THP has no known effect at classic nuclear steroid receptors, but instead is a modulator of the GABAR (Smith et al., 2007).

1.4.2 THP functions as a stress steroid

In addition to its fluctuations at puberty and across the estrous cycle, THP also functions as a stress steroid. In rodents, its levels in brain increase by up to 20-fold after 45 minutes of restraint stress or other forms of stress (CO_2 inhalation) (Higashi et al., 2005; Mukai et al., 2008; Purdy et al., 1991) when decreases in anxiety are observed (Barbaccia et al., 2001). Circulating levels of this steroid are also evidenced in humans after sustained stress associated with performance (Droogleever Fortuyn et al., 2004; Girdler et al., 2006). One potential mechanism for this increase in THP is via CRH and ACTH which have been shown to increase circulating and brain levels of the steroid (Torres et al., 2001), most likely due to activation of adrenal steroidogenesis by ACTH.

2.1 $GABA_A$ receptors

$GABA_A$ receptors (GABARs) mediate most inhibition in the brain (Olsen and Sieghart, 2009). They are pentameric membrane proteins commonly of the form 2α, 2β, and 1 γ (Chang et al., 1990), but many subunit combinations exist from a pool of 6α, 3β, 3γ, δ, ε, θ, π and ρ (Olsen and Sieghart, 2009). These receptors mediate a Cl^- conductance, which is inhibitory in most CNS sites, including hippocampus, after early development (Rivera et al.,

1999) due to increases in the K+-Cl− co-transporter KCC2 which maintains a hyperpolarizing Cl− gradient. GABA is depolarizing in the neonatal rodent CA1 hippocampus (Rivera et al., 1999; Waddell et al., 2011), however, which extends for a longer period in the male due to expression of the N+-K+-Cl−co-transporter NKCC1 (Nunez and McCarthy, 2007), which maintains a depolarizing Cl− gradient. Although other cellular events may underlie this phenomenon (Dzhala et al., 2012), the evidence is as yet inconclusive.

2.1.1 GABAR modulators

The GABAR is recognized as playing a pivotal role in anxiety (Rudolph et al., 1999) and is the target for most anxiety-reducing, sedative drugs (Olsen and Sieghart, 2009), including benzodiazepine, barbiturate and anesthetic drugs as well as for endogenous steroids such as THP (Fig. 1). Early studies established the effect of both isomers, 3α-OH-5α-THP and 3α-OH-5β-THP, which were shown to enhance GABA-gated current in vitro (Callachan et al., 1987; Gee et al., 1987; Harrison et al., 1987; Majewska et al., 1986) and GABA responses in vivo (Smith et al., 1987a), an effect also seen with the parent compound P (Smith et al., 1987b). More recent studies have identified the selective binding site for the steroid for potentiation of current generated by α1β2γ2 (Hosie et al., 2006), which is located in a cavity formed by the α subunit transmembrane domains. A separate binding site was identified for the steroid to directly activate the receptor (Hosie et al., 2006). Behaviorally, THP is anxiolytic (Bitran et al., 1999), anti-convulsant (Frye, 1995) and sedative (Lancel et al., 1997) in adults, as would be predicted for a positive GABA modulator.

2.1.2 GABAR sub-types

The most common GABAR subtype expressed in the brain is $\alpha_1\beta_2\gamma_2$, which expresses subsynaptically (Olsen and Sieghart, 2009), and encounters high concentrations of GABA (~1 mM) released as brief, episodic pulses (<1 ms) from local interneuron terminals (Maconochie et al., 1994). In addition to synaptic expression, however, select GABAR sub-types can also express at non-synaptic sites (extrasynaptically) on the neuron (soma and dendrites) where they underlie a tonic inhibitory current, that is estimated to contribute more to inhibitory tone than the phasic current from synaptic receptors (Bai et al., 2000). These include the $\alpha_5\beta_3\gamma_2$ GABAR, which predominates in the CA1 hippocampal pyramidal cell (Caraiscos et al., 2004; Wisden et al., 1992), and the $\alpha_4\beta\delta$ GABAR, which has highest levels of expression on dentate gyrus granule cells, thalamic relay nuclei and cortical pyramidal cells (Belelli et al., 2005; Chandra et al., 2006; Pirker et al., 2000; Stell et al., 2003; Stell and Mody, 2002; Wisden et al., 1992). The $\alpha_4\beta\delta$ GABAR is unique in that has a high sensitivity to GABA (EC_{50} = ~0.5 μM) (Brown et al., 2002; Sundstrom-Poromaa et al., 2002) and relatively little desensitization (Bianchi et al., 2002) under steady-state conditions, making it well-suited for its extrasynaptic location, where it would come into contact with ambient GABA (~1 μM), maintained by the GABA transporters (Wu et al., 2003). The receptor can desensitize in response to rapid exposure to agonist, however, suggesting that it may not be activated by transmitter spillover (Bright et al., 2011), while receptors distant from the synapse would maintain tonic inhibition supplied by the ambient GABA. This receptor is also unique in that its response to GABA modulatory drugs is distinct from most other GABAR subtypes; it is insensitive to benzodiazepines (Brown et al., 2002; Wieland et al., 1992) and highly sensitive to steroids such as THP and THDOC (Belelli et al., 2002; Bianchi and Macdonald, 2003; Brown et al., 2002; Wohlfarth et al., 2002), which are positive modulators of the receptor. Although conflicting reports exist, they are also sensitive to low concentrations of alcohol in the range which is relevant for social drinking (Sundstrom-Poromaa et al., 2002; Wallner M et al., 2003). In addition, GABA acts as a partial agonist at these receptors, where most modulators, such as THP and THDOC, act by increasing receptor efficacy (Bianchi and Macdonald, 2003; Zheleznova et

al., 2008). In single channel studies, the steroid THDOC increased receptor efficacy by adding a third open state of longer duration to the two open states recorded from the receptor in the absence of steroid (Wohlfarth et al., 2002). Neurosteroids can increase the tonic current recorded from dentate gyrus granule cells (Stell et al., 2003), although some studies have not observed this effect, due to degradation of steroids such as THP and THDOC in this CNS region (Belelli and Herd, 2003). Recent studies suggest that α4 and δ co-express with β2 based on the lack of tonic current in dentate gyrus granule cells in β2 knock-out mice (Herd et al., 2008).

2.1.3 Chloride-dependent effects of THP at $\alpha_4\beta\beta$ GABARs

THP effects at $\alpha_4\beta\delta$ GABARs are unique in that they are dependent upon the direction of the Cl^- current generated by the receptor. Most electrophysiology studies in recombinant receptors record current which is in the depolarizing direction (inward current) due to the ion gradients used. Under these conditions, THP increases current gated by $\alpha_4\beta\delta$ GABARs (Belelli et al., 2002; Bianchi and Macdonald, 2003; Wohlfarth et al., 2002). This effect would be relevant for its effect to increase tonic current in the dentate gyrus, which is depolarizing, but a shunting inhibition (Staley and Mody, 1992). However, THP decreases hyperpolarizing current generated by these receptors (Shen et al., 2007). These effects have been reported in recombinant receptors where either the ion gradient or holding potential were varied, and occurred in the absence of changes in the reversal potential, suggesting that other conductances are not involved (Shen et al., 2007). The effect of THP on hyperpolarizing current generated by $\alpha_4\beta\delta$ GABARs is due to very rapid (<10 ms) acceleration of desensitization (Shen et al., 2007), and is consistent with findings from Macdonald and colleagues who have shown that these receptors desensitize more rapidly when the current is in the hyperpolarizing direction (Bianchi et al., 2002; Haas and Macdonald, 1999), an effect which is accelerated by the steroid (Bianchi and Macdonald, 2003). This is in contrast to the $\alpha5\beta3\gamma2$ GABAR, where desensitization is accelerated when the current is depolarizing (Burgard et al., 1996). This effect of the steroid is dependent upon the positively charged residue arginine 353 in the TM3-TM4 loop (Shen et al., 2007), which may serve as a modulatory site for Cl^-. Modulatory effects of Cl^- have been reported for other GABAR subtypes (Houston et al., 2009; Olsen and Snowman, 1982).

This chloride-dependent effect of the steroid has implications for its ultimate behavioral effect, where the typical effect of the steroid is to increase inhibition and reduce anxiety (Bitran et al., 1999). However, increased expression of $\alpha_4\beta\delta$ GABARs which generate a hyperpolarizing current would decrease inhibition and paradoxically increase anxiety, as has been shown at puberty (Shen et al., 2007).

2.2 Plasticity of the $\alpha_4\beta\beta$ GABARs at puberty

$\alpha_4\beta\delta$ GABARs display a high degree of plasticity even in CNS areas where their expression is normally low, including CA1 hippocampus and midbrain central grey (Pirker et al., 2000; Wisden et al., 1992). Their expression is increased by exposure to the steroid THP (Kuver et al., 2012; Maguire and Mody, 2007; Shen et al., 2005), for periods of 30 min to 48 h, as well as by fluctuating levels of THP produced by exogenous steroid administration or by endogenous cyclicity across the estrous cycle and pregnancy (Griffiths and Lovick, 2005a; Lovick et al., 2005b; Maguire and Mody, 2009; Maguire et al., 2005; Smith et al., 2006).

Our recent studies show that $\alpha_4\beta\delta$ GABAR expression also increases markedly at the onset of puberty in the female mouse (PND ~35–44, assessed by vaginal opening) from almost undetectable levels before puberty (Shen et al., 2007; Shen et al., 2010a). These receptors localize exclusively at non-synaptic (extrasynaptic) sites on the dendritic shaft as well as on the spine, adjacent to the excitatory synapse. Although in the cortex, GABAR expression is

seen on the spine neck of pyramidal cells where it receives direct GABAergic input (i.e., is synaptic) (Kubota et al., 2007; Nusser et al., 1996), this is the first demonstration of a GABAR localized to the spine on a CA1 hippocampal pyramidal cell. Previous reports have shown that GABARs typically express on the soma (80%) and dendritic shaft (20%) of these CA1 neurons (Megias et al., 2001). Expression increased by more than 3 to 8-fold on the dendritic shaft as well as the dendritic spines of CA1 hippocampal pyramidal cells at puberty, which was quantified in the proximal stratum radiatum. The functional expression of this receptor was verified by responses of the pyramidal cells to the GABA agonist gaboxadol at a concentration (100 nM) selective for $\alpha_4\beta\delta$ GABARs (Brown et al., 2002; Meera et al., 2011). Negligible levels of α4 expression are seen on the interneurons in CA1 hippocampus at the onset of puberty, however (unpublished data), which is reflected by the fact that steroid effects on the pyramidal cell are identical whether or not interneuron activity is abolished with the voltage-gated Na+ channel blocker tetrodotoxin (TTX) (Shen et al., 2007).

2.2.1 The direction of GABAergic current in CA1 hippocampal pyramidal cell dendrites

The current generated by dendritic α4βδ GABAR populations on CA1 hippocampal pyramidal cells is hyperpolarizing at puberty. This was determined with direct application of a low concentration of the GABA agonist gaboxadol to the dendrites of a CA1 hippocampal pyramidal cell in recordings using cell-attached mode or gramicidin perforated patch recordings (Shen et al., 2007), which would not disrupt the chloride gradient. Early studies (Alger and Nicoll, 1982) suggesting that the dendritic GABAergic current is depolarizing on CA1 hippocampal pyramidal cells used high concentrations of GABA which would preferentially produce depolarizing responses in low volume systems like the dendrites, but not at the soma, where the larger volume would permit greater adaptive responses. This is likely due to the fact that excessive activation of dendritic GABARs collapses the chloride gradient and permits passage of other ions, such as HCO3−, which would elicit depolarizing responses (Staley and Proctor, 1999), as recently demonstrated (Isomura et al., 2003). In contrast, in the same study (Alger and Nicoll, 1982), dendritic application of the GABA agonist gaboxadol, which would produce less activation of GABARs, generates hyperpolarizing responses on CA1 hippocampal pyramidal cells, as do lower concentrations of GABA itself (Lambert et al., 1991).

2.2.2 THP effects on CA1 hippocampal pyramidal cells at puberty

Because THP exerts chloride-dependent effects at $\alpha_4\beta\delta$ GABARs, the effect of this steroid at puberty would be dependent both upon the increased expression of $\alpha_4\beta\delta$ GABARs on CA1 hippocampal pyramidal cells as well as on the direction of GABAergic current, which is hyperpolarizing at puberty (Shen et al., 2007). Thus, under these conditions, THP acts to reduce the tonic inhibitory current at puberty (Fig. 3) which was a post-synaptic effect because it is observed when action potentials were blocked with TTX in the presence of applied GABA (1 μM) (Shen et al., 2007). THP also increases neuronal excitability at puberty (Fig. 3), assessed both with cell-attached recordings of spontaneous spiking as well as by current clamp recordings (Shen et al., 2007). In the latter, THP reduced the threshold for spiking by increasing the input resistance of the neuron. These paradoxical excitatory effects of the steroid at puberty were not seen in the δ −/− mouse, implicating the increased expression of $\alpha_4\beta\delta$ GABARs at puberty as the mechanism. In contrast, the steroid reduced neuronal excitability before puberty, as expected for its role as a positive GABA modulator at other receptor subtypes where it is known to increase inhibition.

2.2.3 The ultimate effect of THP on CA1 pyramidal cell function at puberty

THP effects at α4βδ GABARs are distinct because lower, physiological concentrations of this steroid (30 nM) would have a greater effect at α4βδ GABARs compared to other

receptor sub-types, where significantly higher concentrations (>100 nM) are required (Belelli et al., 2009). Therefore, at puberty, THP effects (30 nM) on CA1 hippocampal pyramidal neurons would primarily impact the α4βδ GABAR population and the tonic current it generates (Fig. 4A). In addition, recent studies have shown that the GABAergic tonic current contributes a greater net effect on neuronal excitability than does the phasic current (Bai et al., 2000) because it produces a greater total charge transfer than the synaptic current (Fig. 4A). Although GABA is a partial agonist at α4βδ GABARs (Bianchi and Macdonald, 2003), it has greater potency at these receptors, and the 1 μM concentration of ambient GABA would preferentially activate these receptors (Brown et al., 2002). THP increases the input resistance (Rm) by accelerating the desensitization of α4βδ GABARs localized to the dendritic shaft, thus reducing this source of shunting inhibition. (Similar effects have been reported due to the knock-out of α4βδ GABARs (Chandra et al., 2006). This effect occurs within 10 ms (Fig. 2) because fast desensitization is rapid (Jones and Westbrook, 1995), and this is what renders the effect of THP inhibitory. In contrast, synaptic GABARs, such as α1β2γ2, are potentiated only to a modest degree by 30 nM THP (~10%) (Stell and Mody, 2003), but this has less of an impact on neuronal function because the total charge transfer is less than the corresponding change in the α4βδ GABAR population (Fig. 4A). In addition, although tonic current is also generated by α5β3γ2 GABARs (Caraiscos et al., 2004), these are relatively insensitive to THP (Shen et al., 2007) and thus would not be a factor in THP's effects at puberty.

The decrease in the input resistance of CA1 pyramidal cells would permit greater voltage changes produced by both excitatory and inhibitory currents. However, the GABAergic currents in CA1 hippocampus produce a shunting inhibition because the reversal potential for chloride is close to the membrane potential (Xu and Sastry, 2007) and produces less of a driving force on inhibitory currents than exists for excitatory currents (Fig 4B). Therefore, a change in input resistance would have a greater impact on the ability of excitatory currents to generate an action potential (Fig. 4B). Thus, an increase in input resistance would lead to increased neuronal excitability.

2.2.4 Stress steroid-induced anxiety at puberty

The GABAR plays a pivotal role in the generation and modulation of anxiety behavior (Rudolph et al., 1999). Although several brain areas are linked with anxiety behavior, the hippocampus, part of the limbic circuitry which regulates emotion, is one which is directly implicated in THP's effects on anxiety (Bannerman et al., 2004; Bitran et al., 1999). Therefore, one possible mediator of anxiety associated with puberty onset may be linked to the expression of this receptor in CA1 hippocampus. In order to test this possibility, female mice were administered THP (10 mg/kg, i.p.), acutely at puberty onset or before puberty, and tested for anxiety using the elevated plus maze, an animal model of anxiety. THP exerted effects which were opposite in the two groups: it paradoxically increased anxiety behavior at puberty (Fig. 5), but decreased anxiety in the pre-pubertal animals (Shen et al., 2007), similar to what has been reported in adults (Bitran et al., 1999). The anxiety-producing effects of THP were not seen in the δ knock-out mouse, implicating $\alpha_4\beta\delta$ GABARs. The effect of exogenous THP administration was replicated by subjecting the mice to 45 min of restraint stress, which is known to increase release of THP (Higashi et al., 2005). 20 min after the restraint stress, anxiety behavior was reduced in the pre-pubertal mice, but increased in the pubertal mice (Shen et al., 2007). This anxiety-producing effect of the steroid was not seen in the δ −/− mouse and prevented both by finasteride, which prevents formation of THP (Compagnone and Mellon, 2000), as well as by prior administration of 3β-OH-THP, an inactive steroid (Shen et al., 2007), which can prevent effects of THP on GABA-gated current. Taken together, these findings suggest that stress-

induced release of THP produces anxiety at puberty in contrast to its well-established effect to decrease anxiety at other ages (Fig. 6, Summary).

Alternative mechanisms for THP-induced anxiety have been recently presented and include its actions on paraventricular (PVN) hypothalamic neurons which exhibit depolarizing GABA responses during stress when THP can release corticotrophin-releasing hormone (CRH) (Sarkar et al., 2011). This is in contrast to the typical increase in inhibition of these neurons mediated by THP (Belelli et al., 2009), which decreases release of CRH (Patchev et al., 1994). Although depolarizing responses of PVN neurons have not been shown to occur at puberty, these represent another potential mechanism for THP-generated anxiety. However, studies comparing effects of restraint stress in CRH knock-out mice have noted normal behavioral responses despite the lack of CRH release (Dunn and Swiergiel, 1999), suggesting that alternative stress mechanisms exist.

2.3 Paradoxical effects of THP and P on anxiety and dysphoric mood

The paradoxical anxiety-producing effect of THP observed at puberty onset in female mice which is linked with $\alpha_4\beta\delta$ GABAR expression (Shen et al., 2007) has also been observed with P, the parent compound for THP, in human studies at hormonal transitional states. In women with PMDD, negative mood is correlated with administration of E_2 and P after cessation of normal ovarian cyclicity with an analog of luteinizing hormone (Schmidt et al., 1998). This is in contrast to normal women who experience positive mood changes in response to administration of these ovarian hormones. In another study, circulating levels of THP were positively correlated with negative mood systems in women with PMDD (Freeman et al., 2002), again in contrast to asymptomatic controls. In post-menopausal women, ovarian steroids are also correlated with negative mood symptoms when the circulating levels of P and THP are within a normal luteal range (Andreen et al., 2004), suggesting that these paradoxical effects of the steroid may be dose-dependent. Although one study which examined direct effects of THP on the acoustic startle response did not find effects in women with PMDD (Kask et al., 2009), this study did not find anxiety-reducing effects of the steroid in normal women, suggesting that other factors may be required. For instance, in rodent studies the effect of THP on anxiety is most robust in behavioral assays linked with an aversive stimulus (Smith et al., 2006) which was not included in the human study. There are several possibilities for the paradoxical effects of THP in these studies, including potential pre-synaptic effects to disinhibit principal cells, as seen in the periaqueductal grey, when $\alpha_4\beta\delta$ GABARs increase expression on diestrus (Brack and Lovick, 2007; Griffiths and Lovick, 2005b). Although it is not possible to determine potential mechanisms in human studies, the fact that women with PMDD have a decreased response to benzodiazepines (Sundstrom et al., 1997) is consistent with increased expression of $\alpha_4\beta\delta$ GABARs post-synaptically. Benzodiazepine modulation of the GABAR requires a $\gamma 2$ subunit (Sigel, 2002) and an α subunit of the form $\alpha 1$–3,5. GABARs containing the $\alpha 4$ subunit lack a benzodiazepine binding site because of the histidine to arginine substitution at residue 99 in $\alpha 4$ (Wieland et al., 1992).

2.4 Puberty as a THP “withdrawal” state

Recent studies in both the human and mouse report that both P and its neuroactive metabolite THP are also increased during the pre-pubertal period from low levels seen in early development (Fadalti et al., 1999; Mannan and O'Shaughnessy, 1988; McCartney and et al., 2007). However, THP levels drop by 60–70% around the onset of puberty (Fadalti et al., 1999; Mannan and O'Shaughnessy, 1988; Shen et al., 2007) unlike most other steroids including E_2, 17α-hydroxyprogesterone, 20α-dihydroprogesterone and androgens (androstenedione, T) which continue to increase during the pubertal period into adulthood

(Mannan and O'Shaughnessy, 1988). Both ovarian production and hippocampal concentrations of THP were shown to drop precipitously at the onset of puberty in the mouse (Mannan and O'Shaughnessy, 1988; Shen et al., 2007). The decrease in ovarian release of THP around puberty onset was seen after exposure to the precursor steroid pregnenolone, when release of other steroids increased, suggesting that it is due to the relative reduction in activity of synthetic enzymes 5α-reductase and 3α-HSD, rather than due to a reduction in substrate (Mannan and O'Shaughnessy, 1988). Interestingly, the 3β-OH-isomer of THP (3β-OH-THP), which is inactive as a GABA modulator, is released at high levels by the ovary around PND 21 but is reduced to much lower levels of production before PND 29. Because this inactive steroid can block the effect of THP at GABARs (Shen et al., 2007), these findings suggest that the pre-pubertal period (~PND 29–35) may be a time when inhibitory processes in the brain may be enhanced.

This decline in THP ("THP withdrawal") was shown to be responsible for the increased expression of α4βδ GABARs at puberty. Replacement THP (10 mg/kg, i.p. × 3) during pubertal onset prevented the increase in α4βδ expression (Shen et al., 2007), as well as THP's effect to reduce the GABAergic tonic current. This also prevented the paradoxical excitatory effects of THP at this time on CA1 hippocampal pyramidal cell excitability and on anxiety behavior (Shen et al., 2007). THP withdrawal, induced by administration of the 5α-reductase blocker finasteride, has also been shown to increase α4βδ GABAR expression in hippocampus of pre-pubertal mice (Smith et al., 2006), when THP again decreases the tonic inhibitory current generated by these receptors.

Other studies (Griffiths and Lovick, 2005a; Lovick et al., 2005a) have shown that α4βδ expression in interneurons of the periaqueductal grey is increased after P withdrawal or on the stage of late diestrus of the estrous cycle, which follows a peak in circulating levels of P. In this case, increases in tonic inhibition of these interneurons would increase excitability of the output neurons, which are implicated in the circuitry of panic disorder (Lovick, 2000). In both this study and ours, THP withdrawal may represent a model of premenstrual dysphoric disorder (PMDD), which can occur during decreases in circulating levels of P (Cunningham et al., 2009; Rapkin and Winer, 2009), when stress-triggered anxiety and panic attacks are more likely to occur (Nillni et al., 2011; Vickers and McNally, 2004; Yonkers et al., 2008).

THP withdrawal may also be reflected by conditions during the post-partum period when THP levels decline precipitously, and alterations in α4βδ expression occur in dentate gyrus granule cells (Maguire et al., 2009; Maguire and Mody, 2009; Sanna et al., 2009). This change in receptor expression may represent a homeostatic response to maintain normal levels of neuronal excitability.

3.1 Cognition and adolescence: potential sex differences

It has been widely suggested that the onset of puberty may mark the end of a critical period for optimal learning of certain tasks, including motor learning and language acquisition. This has been reported for learning a second language (Johnson and Newport, 1989), including sign language (Newman et al., 2001), and appears to be a more severe boundary for those with pre-existing language deficits (Wright and Zecker, 2004). Although early development may be the most important period for language development, learning a language before puberty preferentially improves ability to detect semantic errors, a fundamental concept in learning (Weber-Fox and Neville, 2001). A number of studies have suggested similar critical periods for optimal cortical reorganization in the visual and auditory systems (Kral et al., 2001; Sharma et al., 2007; Voss et al., 2008) for those with visual and auditory deficits. Interestingly, both structural and functional changes are apparent in musicians who begin training before puberty compared to later learners and non-

musicians (Bailey and Penhune, 2012; Imfeld et al., 2009; Schlaug et al., 1995), suggesting that the pubertal period may have important implications for brain plasticity.

In addition to studies implicating pubertal onset as demarcating the end of a period when CNS plasticity is optimal, other studies have suggested that early adolescence may be a time when learning of certain tasks is transiently impaired. Spatial learning with a computer video game is robust in pre-pubertal children (5^{th} graders) (Subrahmanyam and Greenfield, 1994) and young adults (19–22 year olds) (Pepin and Dorval, 1986a), but not evident in young adolescents (12–13 year olds) (Pepin and Dorval, 1986b; Shavalier, 2004). One prospective study investigated latency to identify verbal and visual mismatches in facial expression across development (McGivern et al., 2002). Clear deficits were evident during early adolescence which recovered by late adolescence. In both of these spatial learning and mismatch detection studies, greater deficits can be seen in females, suggesting a potential gender difference in learning deficits at puberty. A more recent study (Gur et al., 2012) surveying a comprehensive battery of neurocognitive parameters reported global improvement in almost all tasks from age 8 – 21 in both sexes. However, spatial memory declined slightly in 12 – 15 year old females, consistent with pubertal onset, while certain measures of executive control (abstraction) and non-verbal reasoning declined in 12 – 13 year old males, suggesting a sex difference in adolescent performance. Interestingly, for two parameters (spatial memory and non-verbal reasoning), performance for both sexes was highest in 10 – 11 year old children and declined significantly in late adolescence and early adulthood, suggesting that these tasks are optimal before puberty onset. Unfortunately, for many of these studies the use of purely chronological age and the grouping of several age spans may at least partially mask pubertal effects on the measured end-points because the age of puberty onset is variable.

Sex differences in spatial learning have been widely reported, where performance in males is frequently better than for females. Several studies have suggested that these sex differences in spatial memory appear at or after the onset of puberty (Ardila et al., 2011; Gur et al., 2012; Kanit et al., 2000), although there are conflicting reports (Newhouse et al., 2007).

3.1.1 Spatial learning and the hippocampus

Although many CNS targets may contribute to these observed learning deficits in the pubertal period, the hippocampus is one site which plays a pivotal role in spatial learning (Bannerman et al., 2004; Burgess et al., 2002; Maguire et al., 1997) and memory consolidation (Pastalkova et al., 2006). In addition, the hippocampus has more recently been shown to be important for mismatch detection (Kumaran and Maguire, 2006) which is the CNS process which underlies error detection, a critical component of the learning process. Interestingly, hippocampal volume is also correlated with spatial learning during adolescence in humans (Herting and Nagel, 2012), and this parameter can be further correlated with aerobic fitness. These findings suggest the possibility that alterations in hippocampal function may play a role in learning deficits at puberty.

3.1.2 Effect of stress on learning in adolescence

Many studies have considered the effect of stress during adolescence on cognitive function. In general, high levels of chronic stress during adolescence impair cognition (Bellani et al., 2006; Sterlemann et al., 2010), an effect which may be due to the neurodegenerative effects of the stress hormone corticosterone (Watanabe et al., 1992). However, the effect of a stressor at this time would depend upon whether the stress is acute or chronic, the degree of stress and the cognitive state of the individual, as well as the perceived locus of control. In one study (Wolk and Bloom, 1978), middle school children were assessed in their

performance on several academic tasks during stressors (strong environmental interruptions, disturbances and unpredictable obstacles) or no stress conditions. Those students with an internal locus of control exhibited improved performance during stress, while those with an external locus of control had diminished performance as stress level increased. The effect of stress on learning may also be different for simple versus complex learning tasks, as first described by the Yerkes-Dodson law (Yerkes and Dodson, 1908), which describes an inverted U relationship between stress and the learning of simple tasks (Lupien et al., 2007).

In animal studies, acute stress has also been shown to enhance learning during the pubertal period. In one study (Hodes and Shors, 2005), both male and female pubertal rats (35–40 days of age) exhibited improved performance on a trace eyeblink conditioning task 24 h following tailshock compared to unstressed animals. This effect was independent of the estrous cycle in females, and was not seen in pre-pubertal animals, despite robust increases in circulating corticosterone in response to the shock (Hodes and Shors, 2005). Facilitating effects of tailshock stress on learning was also not seen in adult females (Wood and Shors, 1998), suggesting that the pubertal period may represent a unique period for stress effects on cognition. Other studies have also reported facilitating effects of stress on learning in adolescence (Uysal et al., 2012), where a sex difference exists in terms of the shock intensity which produces optimal results. Although CRH and cortisol/corticosterone may be mediators of stress effects in many of these studies, especially those investigating effects of chronic stress, there are reported dissociations between CRH/cortisol release and stress effects (Dunn and Swiergiel, 1999; Hodes and Shors, 2005). Therefore other systems may also play a role in stress effects on learning during the pubertal period.

3.2 $GABA_A$ receptors and learning

Many studies have shown that drugs which act as positive modulators of the GABAR impair learning in adults. It is well known that benzodiazepine tranquilizers are amnestic, as are anesthetics such as propofol (Veselis et al., 2009). Both alcohol and the stress steroid THP impair spatial learning on the Morris Water Maze (Matthews et al., 2002). In the adult, GABARs containing the α5 subunit are the predominant extrasynaptic inhibitory receptor on the dendritic shaft (Brunig et al., 2002). Genetic knock-out or pharmacological knock-down of this receptor prevents learning impairment produced by the anesthetic etomidate (Cheng et al., 2006), and enhances a number of learned behaviors including fear conditioning (Chambers et al., 2003; Collinson et al., 2002; Crestani et al., 2002). These effects of positive GABA modulators are also evidenced in in vitro models of synaptic plasticity, including long-term potentiation (LTP). LTP can be reduced by a wide variety of these GABA modulators, including alcohol, THP and propofol (Izumi et al., 2005; Nagashima et al., 2005; Tokuda et al., 2011), suggesting a potent effect of inhibition in the hippocampus in limiting synaptic plasticity.

3.3 α4βδ GABARs and synaptic plasticity at puberty

The emergence of α4βδ GABARs on dendritic spines, as well as on the dendritic shaft, at puberty (Shen et al., 2010a) suggests a role for these receptors in regulating cognition during adolescence, because the dendritic spine is the CNS site for induction of synaptic plasticity. In adult CA1 hippocampus, GABARs localize to the soma (80%) or the dendritic shaft (20%) (Megias et al., 2001), where they can either be sub-synaptic or extrasynaptic, but they are not localized to the dendritic spine, in contrast to cortex where GABAergic innervation of spines is commonly found (Kubata et al., 2007). The predominant extrasynaptic GABARs in the adult are of the subtype α5β3γ2 (Brunig et al., 2002). However, at puberty (~PND 35), extrasynaptic α4βδ expression increases markedly from nearly undetectable levels (Shen et al., 2010a): the proportion of spines which are immunolabeled with α4 and δ is

increased by 3 to 6-fold, respectively, while the immunoreactivity per spine is increased 4 to 8-fold, respectively, as determined with silver-intensified immunogold (SIG) labeling and electron microscopy in the proximal stratum radiatum (Fig. 7). It is estimated that 25% of spines may express α4βδ GABARs (Aoki et al., 2012) based on the analysis of immunolabeling. Similar increases in immunolabeling are seen on the dendritic shaft. Pubertal expression of α4βδ GABARs in these dendritic compartments is a transient event, and is reduced significantly by about PND 44 (Aoki et al., 2012), suggesting that high expression of extrasynaptic GABARs in the dendrites persists for a period of about 10 d.

α4βδ GABARs activated by ambient GABA produce a tonic inhibition in hippocampus at adolescence (Shen et al., 2007). At this time, activation of NMDA receptors is impaired (Shen et al., 2010a) most likely because the shunting inhibition produced by α4βδ receptors reduces the depolarization necessary for Mg+ unblock of the receptor (Herron et al., 1986). As a consequence, induction of long-term potentiation (LTP), an in vitro model of learning, produced by stimulation of the Schaffer collaterals to CA1 hippocampus with theta burst stimulation is nearly prevented (Shen et al., 2010a). This deficit in synaptic plasticity is not seen with total GABAR blockade or in the δ knock-out mouse, implicating α4βδ GABARs as the mediating factor. In contrast, LTP induction is robust in the hippocampus of pre-pubertal mice. Interestingly, selective blockade of only the synaptic GABARs with 200 nM gabazine does not facilitate LTP induction at puberty, suggesting that the deficit is solely due to the extrasynaptic GABAR population (Shen et al., 2010a). These findings are consistent with an earlier report suggesting that LTP induction is impaired in adolescence due to an increase in GABAergic inhibition (Meredith et al., 2003), although puberty onset and α4βδ were not identified. In contrast, activation of NMDA current is robust in the δ knock-out or after total GABAR blockade (Shen et al., 2010a), suggesting that the deficit in synaptic plasticity at puberty is due to increases in GABA inhibition rather than due to deficits in NMDA receptor expression.

3.3.1 E_2 and α4βδ GABAR expression

The onset of puberty in the female is preceded by increases in circulating levels of the ovarian steroid E_2, which in the mouse increase two-fold 7–8 days before vaginal opening (Ahima et al., 1997) and in the human appear well before menarche in the pre-pubertal period (Janfaza et al., 2006). Unlike P and THP, E_2 levels continue to increase throughout the pubertal period (Ahima et al., 1997; Janfaza et al., 2006), but do not fluctuate (Hodes and Shors, 2005) despite changes in the morphology of cells in the vaginal lavage. The increase in α4βδ GABAR expression at puberty may also be a result of the high levels of circulating E_2 because this steroid has been shown to facilitate expression of α4βδ GABARs (Shen et al., 2005). E_2 acts via specific nuclear receptors, ERα and ERβ, which govern gene transcription of specific proteins, and these are localized both to the dendritic shaft and spine of hippocampal neurons of female rats and mice (Mitterling et al., 2010). Expression of ERα and ERβ is highest in spines of the CA1 hippocampal pyramidal cells (Mitterling et al., 2010), with ERα most prevalent in the proximal region of the stratum radiatum where expression of α4 and δ has been shown to increase at puberty (Shen et al., 2010a). In fact, expression here is 3 to 4-fold higher than observed in the dentate gyrus (Mitterling et al., 2010) where α4βδ GABAR expression is normally high (Benke et al., 1997; Wisden et al., 1992), suggesting the possibility that E_2 may function as a modulator of receptor expression to a greater extent in CA1 hippocampus where α4βδ expression is highly plastic (Smith et al., 2007). Although its role in α4βδ expression is not yet known, E_2 increases brain derived neurotrophic factor (BDNF) (Jezierski and Sohrabji, 2003; Sato et al., 2007; Scharfman et al., 2003) which has also been shown to increase activity of the α4 promotor (Roberts et al., 2006) as well as trafficking of δ to the surface membrane (Joshi and Kapur, 2009).

3.3.2 Hippocampal-dependent spatial learning, α4βδ GABARs and adolescence

It is well-known that the hippocampus is the primary site for spatial learning (Bannerman et al., 2004; Burgess et al., 2002; Pastalkova et al., 2006). It would thus be expected that the shunting inhibition produced by α4βδ GABARs on dendritic spines of CA1 pyramidal cells at puberty would impair spatial learning, as was observed. This was determined using a hippocampal-dependent spatial learning task employing active avoidance (Cimadevilla et al., 2001) which is dependent upon LTP (Pastalkova et al., 2006): for the task, mice are trained across three 10-min sessions to avoid a sector on a rotating platform which delivers a mild shock (~0.2 mA). The shock sector is stationary with respect to the room, but rotates with the platform, so that the mouse must move in order to avoid the shock (Fig. 10). (The degree of shock is sub-threshold for stress hormone release (Friedman et al., 1967) suggesting that this is a relatively unstressful task compared to other animal models of learning (Harrison et al., 2009).) For each trial, the latency to enter the shock sector is a measure of learning. Under these conditions, pubertal mice have impaired learning compared to pre-pubertal mice, because they fail to reach criterion (120 s latency to enter the shock sector) after 7 trials (Fig. 10), in contrast to the pre-pubertal mice who reach criterion in 2.5 trials (with a mean latency of 275 s) (Shen et al., 2010b). This learning deficit is not seen in δ knock-out mice at puberty, implicating α4βδ GABARs (Shen et al., 2010a).

3.3.3 The effect of the stress hormone THP on synaptic plasticity at puberty

Because THP reduces hyperpolarizing current at α4βδ GABARs, its increased expression at puberty decreases the shunting tonic inhibition of CA1 hippocampal pyramidal cells (Shen et al., 2010a). This effectively removes the impediment to activation of NMDA receptors, and permits robust activation of NMDA current (Fig. 8), which is also correlated with reversal of the deficit in induction of LTP (Fig. 9), an NMDA receptor-dependent event (Herron et al., 1986). The effect of THP on LTP induction is observed when the steroid is restricted to the dendrites of the stratum radiatum during theta burst stimulation, suggesting that it facilitates LTP exclusively by facilitating induction (Shen et al., 2010a), rather than maintenance of LTP. This was confirmed by a study where THP was applied before induction, washed out and re-applied 5 min after theta burst stimulation, when it did not facilitate LTP. The facilitating effect of THP on LTP was not seen in the δ knock-out, suggesting that α4βδ GABARs are the target for this steroid's effect on synaptic plasticity at puberty (Shen et al., 2010a).

In a similar manner, THP facilitates spatial learning using the hippocampal-dependent active avoidance task (Shen et al., 2010a). After THP administration, the pubertal mice reach criterion in less than 3 trials, with a mean latency of 250 s (Fig. 10), in contrast to their learning deficit in the absence of THP. Facilitating effects of THP on learning are not seen in the δ knock-out mouse, again suggesting that this receptor is the target for THP at puberty (Fig. 11, summary).

3.4 α4βδ GABARs, puberty and eating disorders

Anorexia nervosa and bulimia are eating disorders with restricted or maladaptive patterns of food intake which most commonly begin in adolescence (Kaye, 2008). The disorder is most prevalent in females where the female:male ratio is 7:1 (Kaye, 2008). It has been estimated that, depending on the source, 2 – 7% of adolescent females in the United States have experienced an eating disorder (Kaye, 2008), compared with a 0.5% prevalence in the whole population. Although these disorders can potentially have fatal outcomes (Birmingham et al., 2005), treatment options are limited (Powers and Bruty, 2009). A number of studies have suggested that monoamine disturbance may contribute to these disorders, but the treatment

outcomes are frequently equivocal (Powers and Bruty, 2009). One recent study (Aoki et al., 2012) has reported increased expression of $\alpha_4\beta\delta$ GABARs in CA1 hippocampus with an activity-based animal model of anorexia nervosa (ABA). In this model, female rats are conditioned during puberty with both food restriction and running wheel activity to mimic conditions underlying anorexia in humans. Expression of α_4 and δ on spines of CA1 hippocampal pyramidal cells of control female rats increases at the onset of puberty (PND 32–36), as it does in mice, and remains elevated for about 10 d, declining around PND 42 (Aoki et al., 2012). However, α_4 and δ expression remains elevated on dendritic spines of ABA rats, such that, by PND 44, expression is 2 – 6-fold higher than corresponding levels of expression in the control animals (Aoki et al., 2012). In contrast, animals which experience only food restriction or running wheel activity alone do not show increased $\alpha_4\beta\delta$ expression on hippocampal spines. This prolonged period of increased expression of $\alpha_4\beta\delta$ GABARs on spines has implications for both stress-triggered anxiety and cognitive impairment, as already shown for pubertal female mice (Shen et al., 2007; Shen et al., 2010b). In fact, studies in humans show extensive co-morbidity of anxiety disorders with anorexia nervosa and bulimia (Kaye et al., 2004; Swinbourne et al., 2012). In addition, a recent study has reported decreased cognitive ability in adolescents with anorexia (Andres-Perpina et al., 2011), which is correlated with hyperactivity in several brain regions, but predominantly in the temporal lobe, during a working memory task (Castro-Fornieles et al., 2010). These findings suggest that increased activity in this region may be required to attain normal levels of working memory. The findings are also consistent with increased levels of shunting inhibition in hippocampus in anorexia which would require greater depolarization to activate NMDA receptors, as we have shown in pubertal mice when expression of $\alpha_4\beta\delta$ GABARs increases on dendritic spines of CA1 hippocampus (Shen et al., 2010a). Taken together, these findings suggest a link between anxiety and cognitive deficits and eating disorders, which is consistent with increased expression of $\alpha_4\beta\delta$ GABARs.

4.1 Conclusions

The onset of puberty demarcates a vulnerable period when mood changes occur and certain cognitive processes can be compromised. The many hormonal events which converge at the onset of puberty may contribute to these changes in mood and cognition that characterize early adolescence. However, the emergence of α4βδ GABARs during early adolescence play a pivotal role in these changes, and act as a target for one stress steroid which can trigger anxiety and alter cognitive processes (Summary Figs. 4,6,11). The outcomes resulting from altered stress responses at puberty are known to contribute to psychopathologies later in development, suggesting that unraveling the cellular mechanisms for these events is an important avenue of investigation.

Abbreviations

ABA	activity-based animal model of anorexia nervosa
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CRH	corticotrophin releasing hormone
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
E_2	17β-estradiol (an estrogen)

EPSC/EPSP	excitatory post-synaptic current/potential
ER	estrogen receptor
fMRI	functional magnetic resonance imaging
GABA	γ-amino-butyric-acid
GABAR	$GABA_A$ receptor
3α-HSD	3α-hydroxysteroid oxidoreductase
i.p.	intraperitoneal
KCC2	K+-Cl− co-transporter
LTP	long-term potentiation
NKCC1	Na+-K+-Cl+ co-transporter
NMDA	N-methyl-D-aspartate
P	progesterone
PMDD	premenstrual dysphoric disorder
PND	post-natal day
PVN	paraventricular nucleus (hypothalamus)
SIG	silver-intensified gold
T	testosterone
TBS	theta-burst stimulation
TEA	tetra-ethylammonium
THDOC	3α,21-dihydroxy-5α-pregnan-20-one
THP	3α-OH-5[α]β-pregnan-20-one or [allo]pregnanolone
TTX	tetrodotoxin

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Highlights

- Puberty onset increases hippocampal expression of α4βδ GABARs.
- The stress steroid THP decreases hippocampal tonic inhibitory current at puberty.
- THP effects α4βδ GABARs are dependent on the chloride gradient.
- THP and restraint stress produce greater anxiety at puberty.
- α4βδ expression on CA1 pyramidal cells at puberty reduces synaptic plasticity.
- α4βδ GABARs play a role in mood and cognition at puberty in female mice.

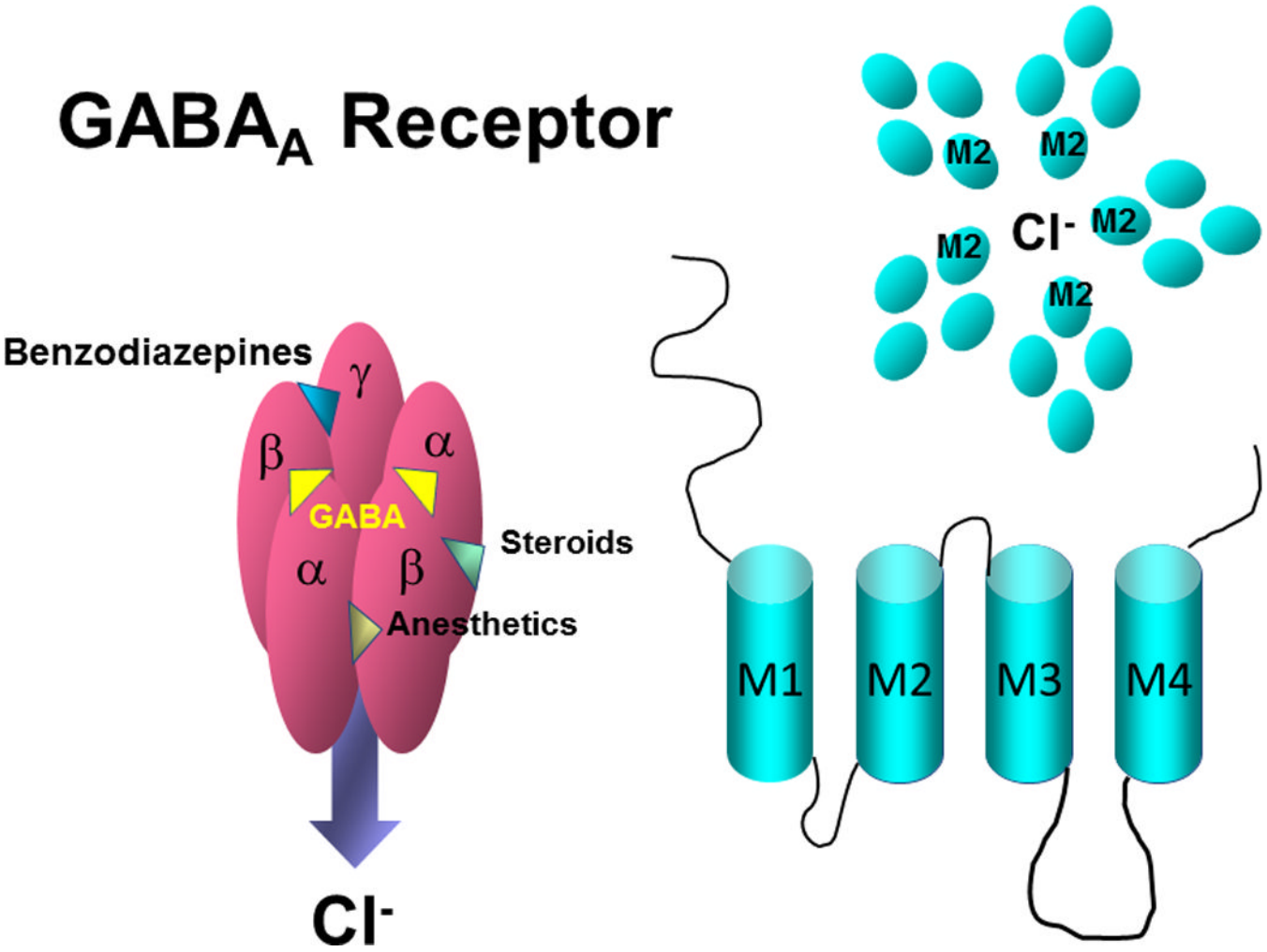

Figure 1. The GABA$_A$ receptor

The GABA$_A$ receptor (GABAR) is a pentameric membrane protein typically composed of 2α, 2β and 1γ or δ subunit, but other possibilities exist from a pool of 6α, 3β, 3γ, δ, ε, θ, π and ρ. Each subunit is composed of 4 transmembrane spanning helices (M1–M4); M2 lines a central Cl− channel. The intracellular loop between M3 and M4 is 2-fold longer in α4 compared to α1, with very low homology. Binding of 2 GABA molecules (yellow) between α and β gates a Cl− current which can be increased by a variety of compounds including sedative drugs (benzodiazepines, anesthetics, alcohol) and the endogenous steroid THP. Benzodiazepines bind between α (α1–3 or 5) and γ, but do not bind to α4-containing GABARs.

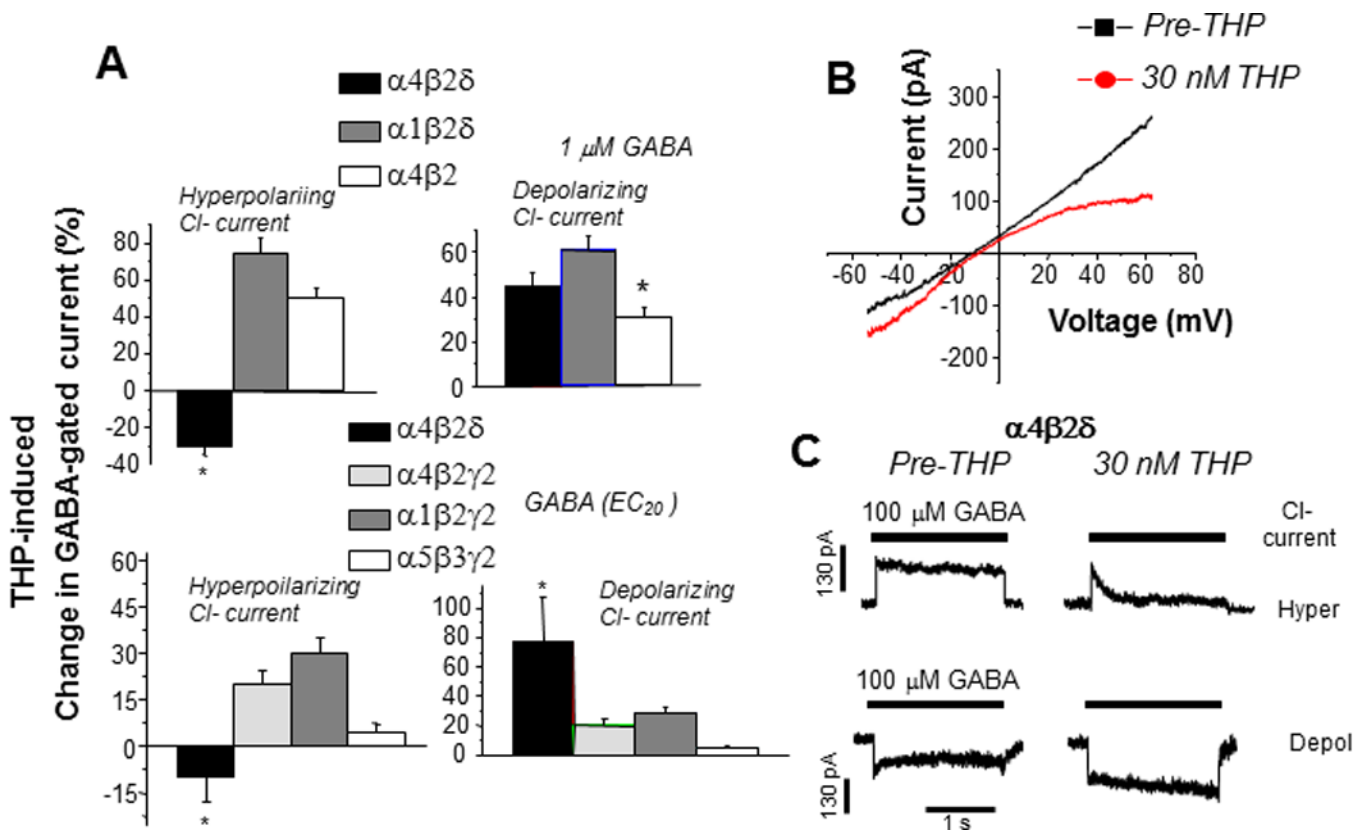

Figure 2. Chloride-dependent effects of THP at α4βδ $GABA_A$ receptors

The neurosteroid THP decreases hyperpolarizing current gated by α4βδ $GABA_A$ receptors transiently expressed in HEK-293 cells and recorded with whole cell voltage clamp techniques. A. Mean effects of THP on hyperpolarizing and depolarizing currents in response to 1 μM GABA (upper panel) or the GABA EC_{20} (lower panel; α4β2δ, 0.1 μM; α4β2γ2, 5 μM; α1β2γ2, 10 μM; α5β3γ2, 5 μM) from 6–7 cells for each group (*P = 0.05 versus the other receptor subtypes). n= 6 – 8 cells/group. B. Effects of 30 nM THP on current generated by a voltage ramp over 400 ms where the ECl = 0 mV. THP increases depolarizing current (voltage < 0 mV) but decreases hyperpolarizing current (voltage > 0 mV). (Leak-subtracted current is presented as the average of three traces). n=5 – 6 cells/ group. C. Effects of THP on desensitization of hypepolarizing (upper traces) and depolarizing (lower traces) current at α4βδ receptors (representative of 6 cells per group). THP accelerates desensitization of the hyperpolarizing current. (Used with permission (Shen et al., 2007).)

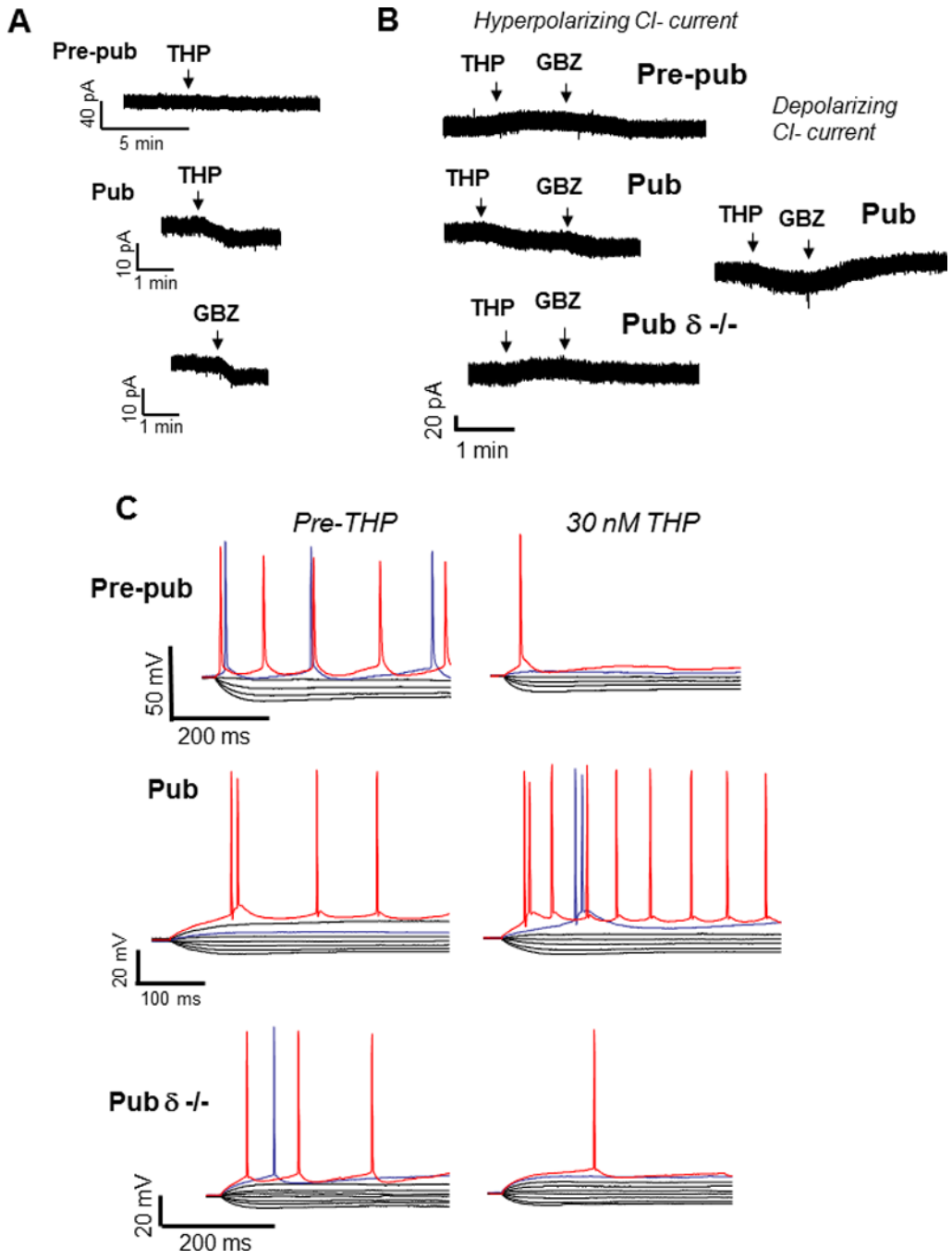

Figure 3. THP effects on CA1 hippocampal pyramidal cells at puberty

THP reduces the tonic GABAergic current and increases excitability of CA1 hippocampal pyramidal cells at puberty. A. THP effects on the tonic current recorded from CA1 hippocampal pyramidal cells in the slice using gramicidin perforated patch techniques to maintain the internal Cl− milieu. 1 μM TTX, 1 μM GABA and 2 mM kynurenic acid were added to isolate the GABAergic post-synaptic component. L-655, 708, CGP55845 and TEA were also added to block α5 and $GABA_B$ receptors and K+ channels, respectively. Pre-pub, pre-pubertal; Pub, pubertal; GBZ, gabazine, a GABA antagonist (presented for comparison). THP reduces the current in Pub slices. (Representative of 5 cells/group) B. Hyperpolarizing current recorded from CA1 hippocampal pyramidal cells in the slice using whole cell patch

clamp techniques (ECl = –70 mV, –50 mV holding potential; pipette solution, K-gluconate; bath, 200 nM gabazine to block synaptic current and 2 mM kynurenic acid to block excitatory current). Inset, effects of THP on the depolarizing tonic current at puberty (ECl = −30 mV, pipet solution, CsCl). THP reduces hyperpolarizing current in wild-type but not δ knock-out mice, but potentiates depolarizing current. (Representative of 8 – 12 cells/group) C. Whole-cell current-clamp recordings reveal voltage responses recorded in response to increasing 0.3-nA current injection (initial current −1 nA). (The THP trace lacks the 800-pA current trace for ease of comparison.) THP lowers the current threshold for spiking of pyramidal cells at the onset of puberty in wild-type but not δ knock-out mice. Red trace, equivalent current injection, threshold for the less excitable state. Blue trace, equivalent current injection, threshold for the more excitable state. (Representative of 7 – 8 cells/group) (Revised and used with permission (Shen et al., 2007).)

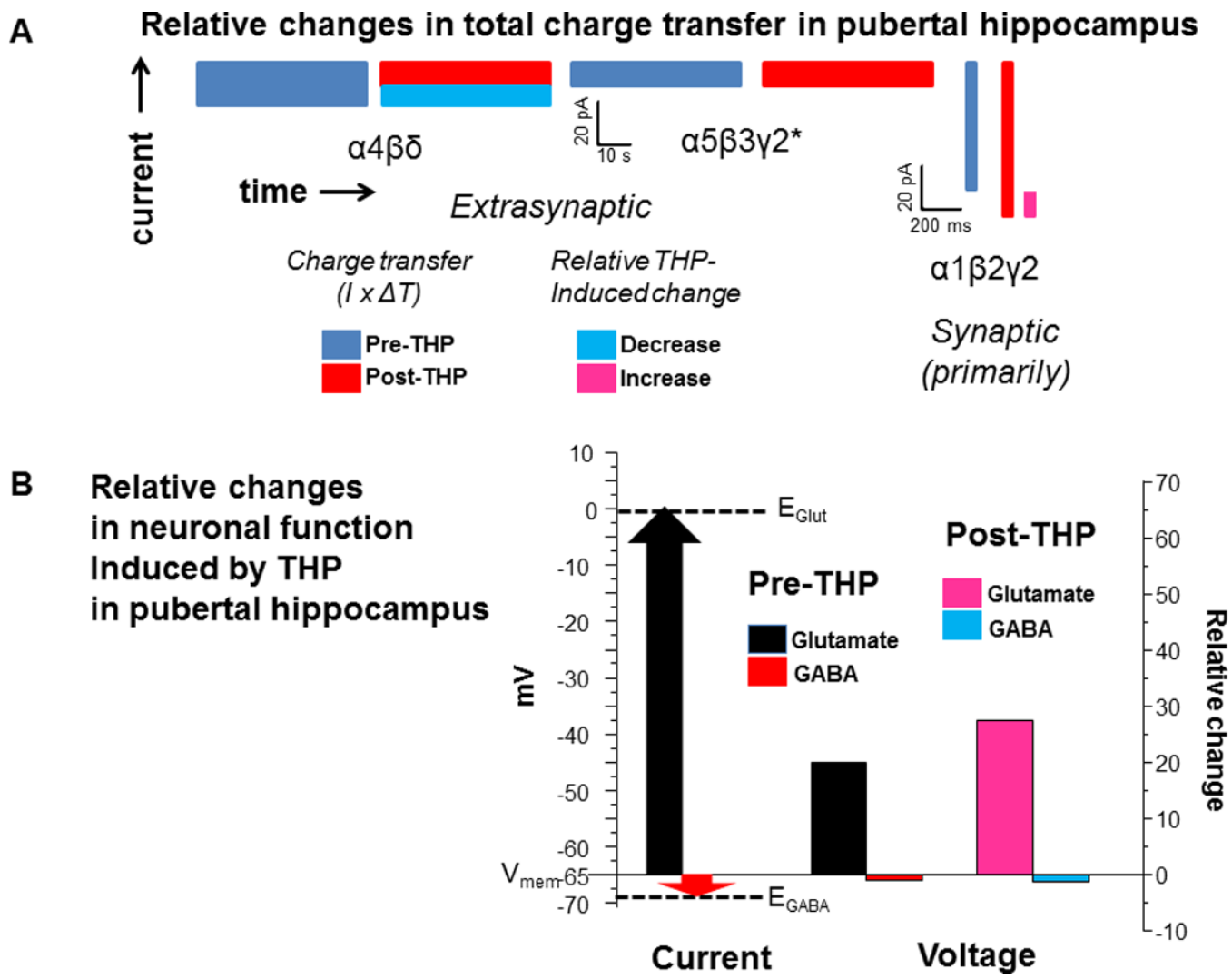

Figure 4. Relative changes in the total charge transfer and neuronal function produced by THP in pubertal hippocampus

A, total charge transfer. Estimates of charge transfer based on average values obtained from whole cell patch clamp recordings of CA1 hippocampal pyramidal cells in the slice, before and after 30 nM THP across a 60 s time period (boxes indicate approximate total charge transfer: current (y axis) by total time (x axis). α4βδ, 20 pA tonic current × 60,000 ms; α5β3γ2, 10 pA tonic current × 60,000 ms; α1β2γ2, 60 pA, phasic current × 3600 ms (5 ms half-width × 12 Hz frequency). THP decreases the tonic current by 45% at puberty when α4βδ expression is high, has no effect on α5β3γ2 (*based on results from pre-pubertal mice, where α5β3γ2 represent the majority of the tonic current) and increases the half-width of the phasic current by ~10% (likely to represent α1β2γ2 GABARs). Therefore, the relative decrease in total charge transfer produced by THP would be approximately 20-fold greater for the tonic current compared to the corresponding increase in total charge transfer produced by THP on the phasic current. These values are similar to what has been reported for anesthetic effects (Bai et al., 2000). B, neuronal function. Y axis (left), Voltage (mV) indicated for the membrane potential (V_{mem}), reversal potential for glutamate (E_{Glut}, dashed line) and reversal potential for GABA (E_{GABA}, dashed line) for a typical mature CA1 hippocampal pyramidal cell. Because V_{mem} values are considerably further from E_{Glut} compared to E_{GABA}, opening of the channels on these receptors produces greater excitatory

current relative to inhibitory GABAergic current (arrows). Y axis (right), relative changes in current and the resulting change in voltage. After 30 nM THP, there is a ~37% increase in input resistance (Shen et al., 2007). This produces a greater increase in the depolarization produced by glutamate (Glut) relative to the negligible decrease in hyperpolarization produced by GABA, calculated using Ohm's Law. Thus, THP has an excitatory effect at puberty.

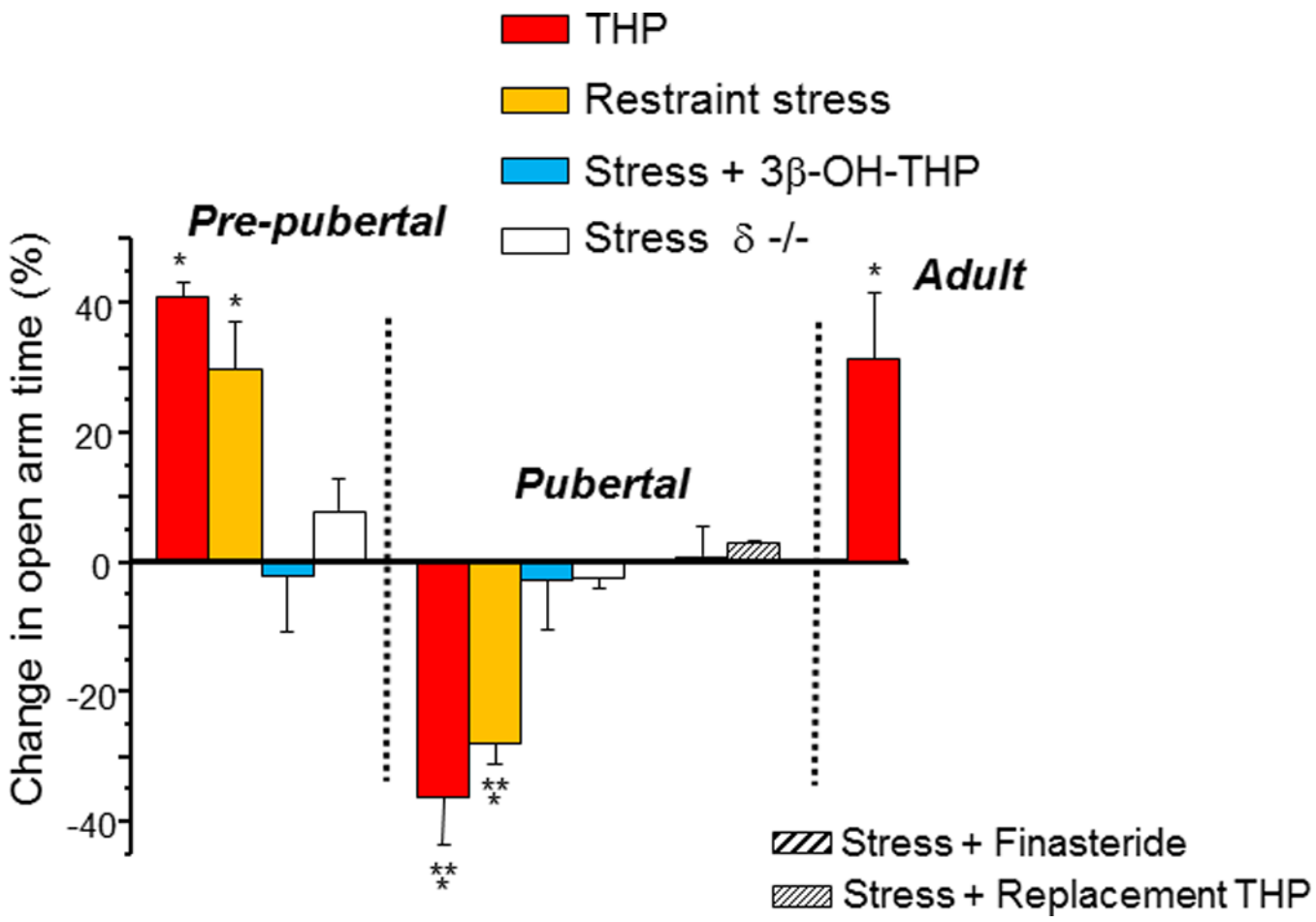

Figure 5. THP paradoxically increases anxiety after the onset of puberty

Alterations in anxiety produced by restraint stress or injection of THP (10 mg/kg, i.p.) are presented as a percentage change in open arm time in the elevated plus maze compared to mean values from a sham control group, age-and genotype-matched to the experimental group. To test the role of THP release in the stress response, in some cases the inactive 3β-OH isomer of THP (stress + 3β-OH-THP) or finasteride (a 5α-reductase inhibitor) were preadministered. Replacement THP (10 mg/kg, i.p., in oil, for 3 d) was also administered to prevent the decline in THP at puberty. n = 6 – 9 mice for each group. *P = 0.05 versus control, **P = 0.05 versus Pre-pub. (Used with permission (Shen et al., 2007).)

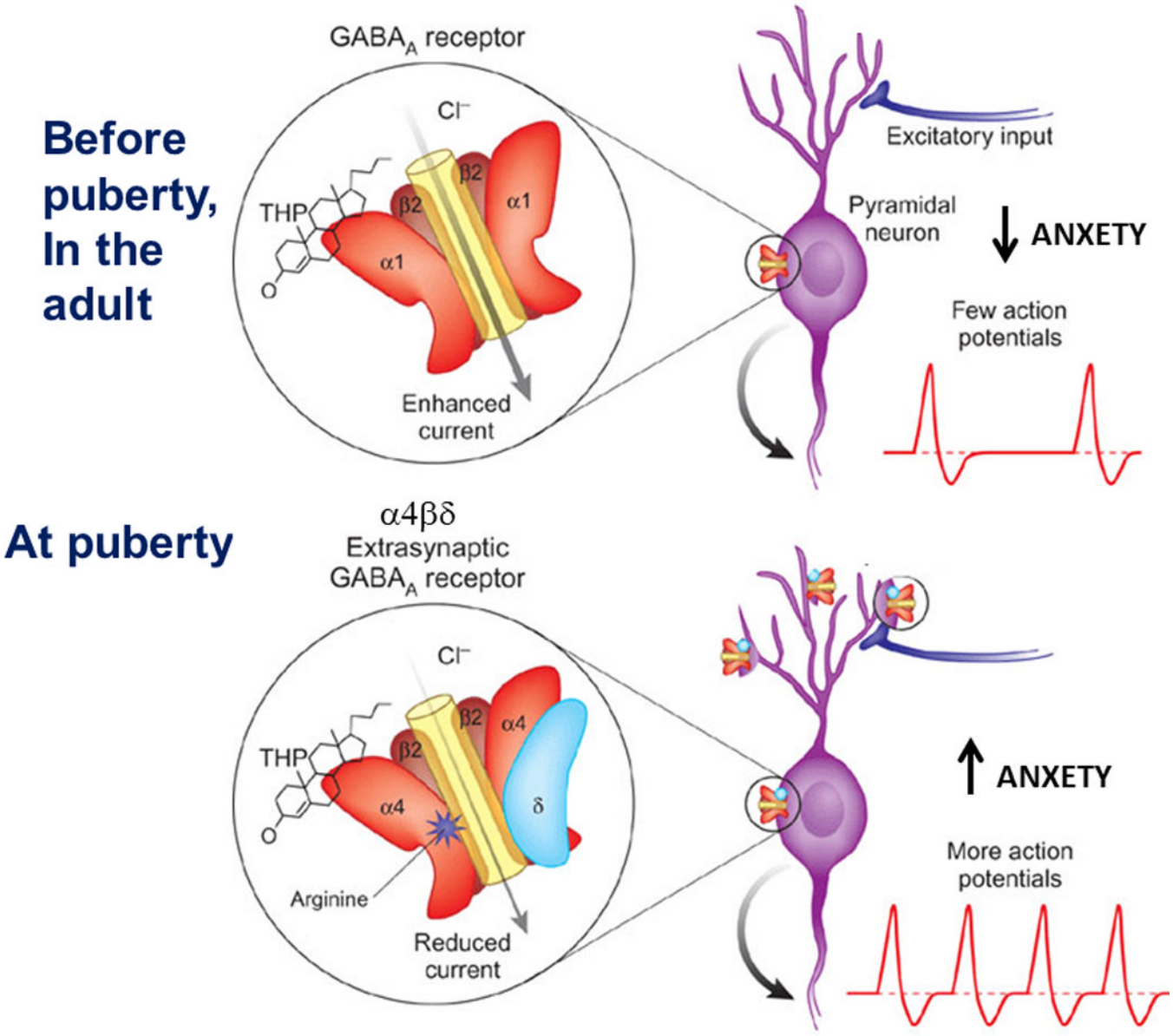

Figure 6. Summary of the effects of the stress steroid THP on CA1 hippocampal pyramidal cells at puberty

Before puberty and in the adult (upper panel) α4βδ GABAR expression is low. THP potentiates non- α4βδ GABARs, which would reduce the likelihood of action potentials. During the pubertal period (lower panel), however, α4βδ GABAR expression increases on the dendrites and spines of the pyramidal cells (lower panels). Current from these receptors is reduced by THP, which would increase neuronal excitability, increasing the likelihood of action potential generation. This effect of the stress steroid is linked both to increased anxiety, which may be a result of its effects at the α4βδ GABAR. (Revised and used with permission (McCarthy, 2007).)

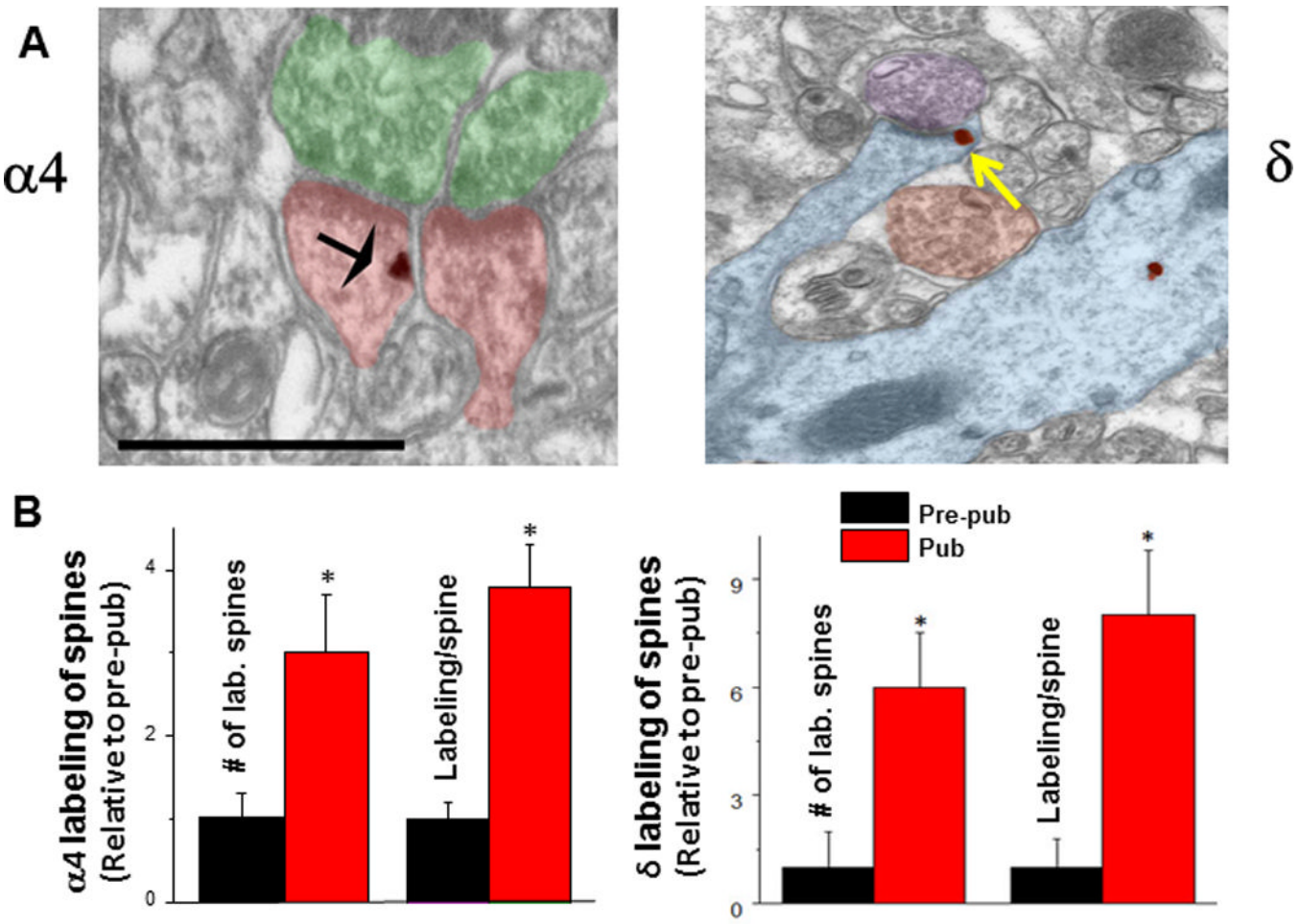

Figure 7. α4 and β GABA$_A$ receptor subunit expression increases on dendritic spine membranes of CA1 hippocampal pyramidal cells at puberty

A. α4 (left) and δ (right) silver-intensified immunogold labeling (SIG) occurs along the plasma membrane of spines forming excitatory synapses. α4, black arrow; δ, yellow arrow. Scale, 100 μm. Shafts also exhibit immunoreactivity. B. Averaged data. # of labeled spines (α4, *P < 0.018; δ, **P = 0.002) and labeling per spine (α4, *P < 0.005, δ, **P = 0.00091) increase at puberty (Pub) relative to pre-puberty (Pre-pub). n = 50–80 spines.

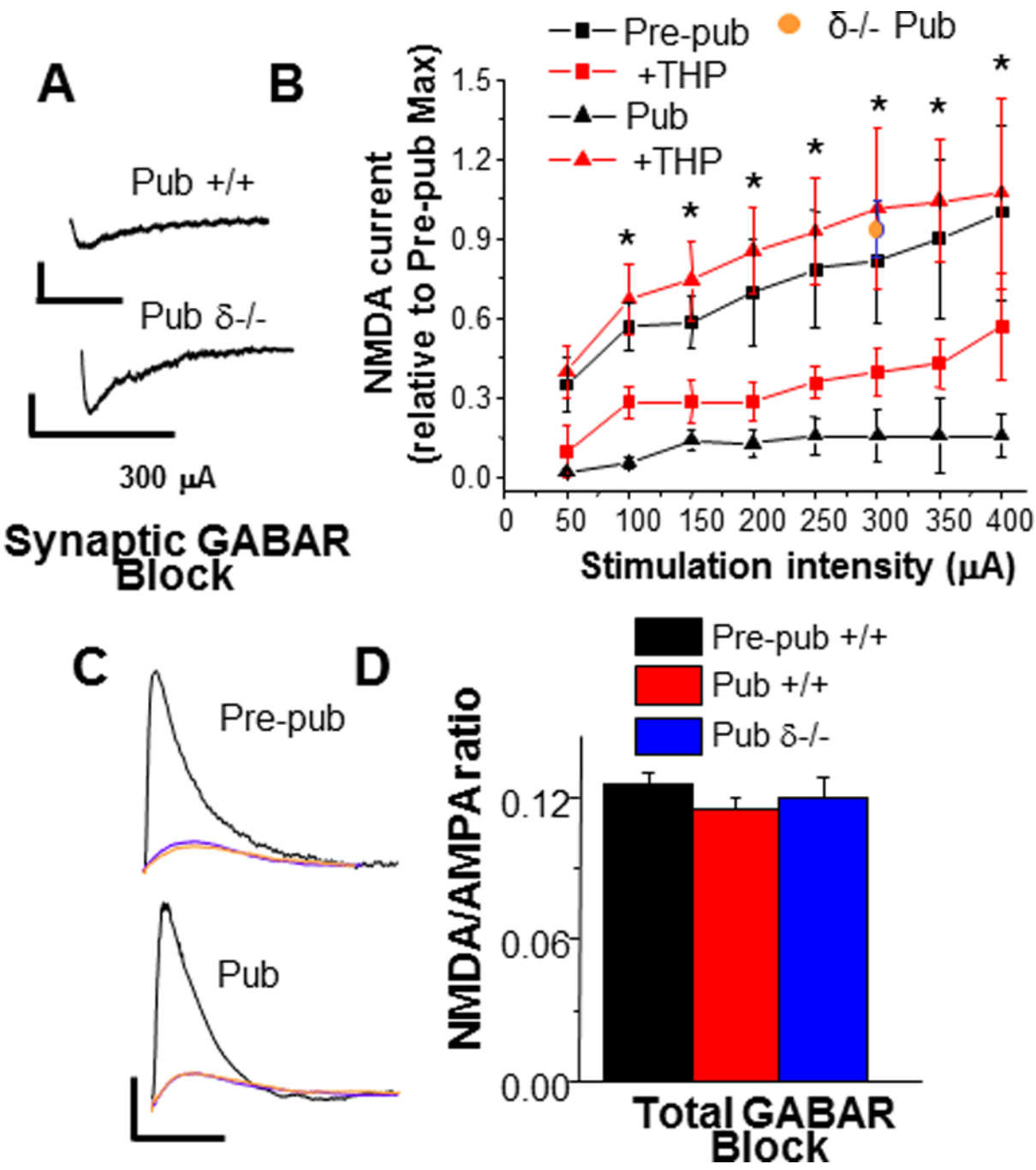

Figure 8. NMDA current is decreased at puberty in CA1 hippocampal pyramidal cells: reversal by THP and GABAR blockade

A. Representative traces, evoked (300 μA) NMDA current (0.05 Hz; +/+, above; δ −/−, below) recorded at −60 mV (1 mM $MgCl_2$, bath) with synaptic GABAR blockade (200 nM gabazine) but extrasynaptic GABAR current intact. Scale for +/+, 20 pA, 100 ms; for δ −/−, 20 pA, 250 ms. B. NMDA EPSC amplitude with increasing stimulation intensities (Pre-pub, black squares; +THP, open squares; Pub, black triangle; +THP, open triangle; Pub δ−/−, orange). Amplitudes decreased at puberty, but were restored by THP and δ knock-out.*P < 0.05, Pre-pub versus Pub. C, D: Representative traces and summary data of evoked EPSPs (whole-cell current clamp, black) and NMDA EPSPs (blue; +THP, red) with total GABAR

blockade. Scale, 1mV, 100 ms. The NMDA/AMPA ratio was unchanged with total GABAR blockade, suggesting that NMDA receptor expression is not altered by the onset of puberty. (*P < 0.05 versus all other groups, **P < 0.05 versus Pre-pub, Pub). n= 7 – 8 cells/group. (Revised and used with permission (Shen et al., 2010a).)

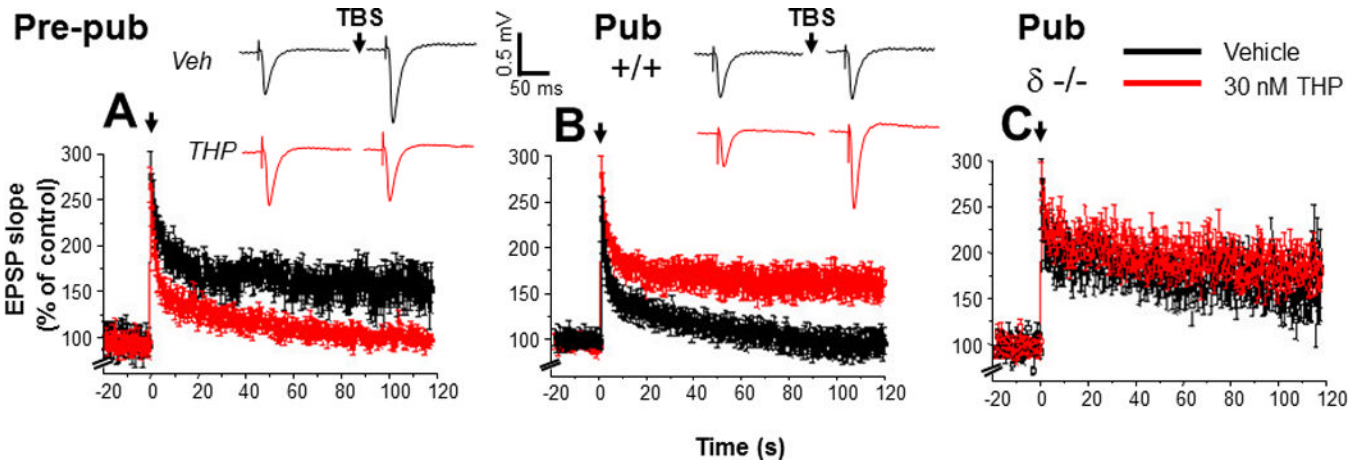

Figure 9. LTP induction is attenuated at puberty: reversal by the stress steroid THP and β knock-out

Theta burst stimulation (TBS - 0 time) induced LTP (black) before puberty (Pre-pub, A) but not in the pubertal (Pub, B) CA1 hippocampus. THP (red, 30 nM) permitted LTP induction at puberty. (Inset, Representative field EPSPs. TBS, arrow. Scale, 0.5 mV, 50 ms.) C. LTP, Pubertal δ−/−, where TBS induced LTP. n=6 – 9/group. (Used with permission (Shen et al., 2010a).)

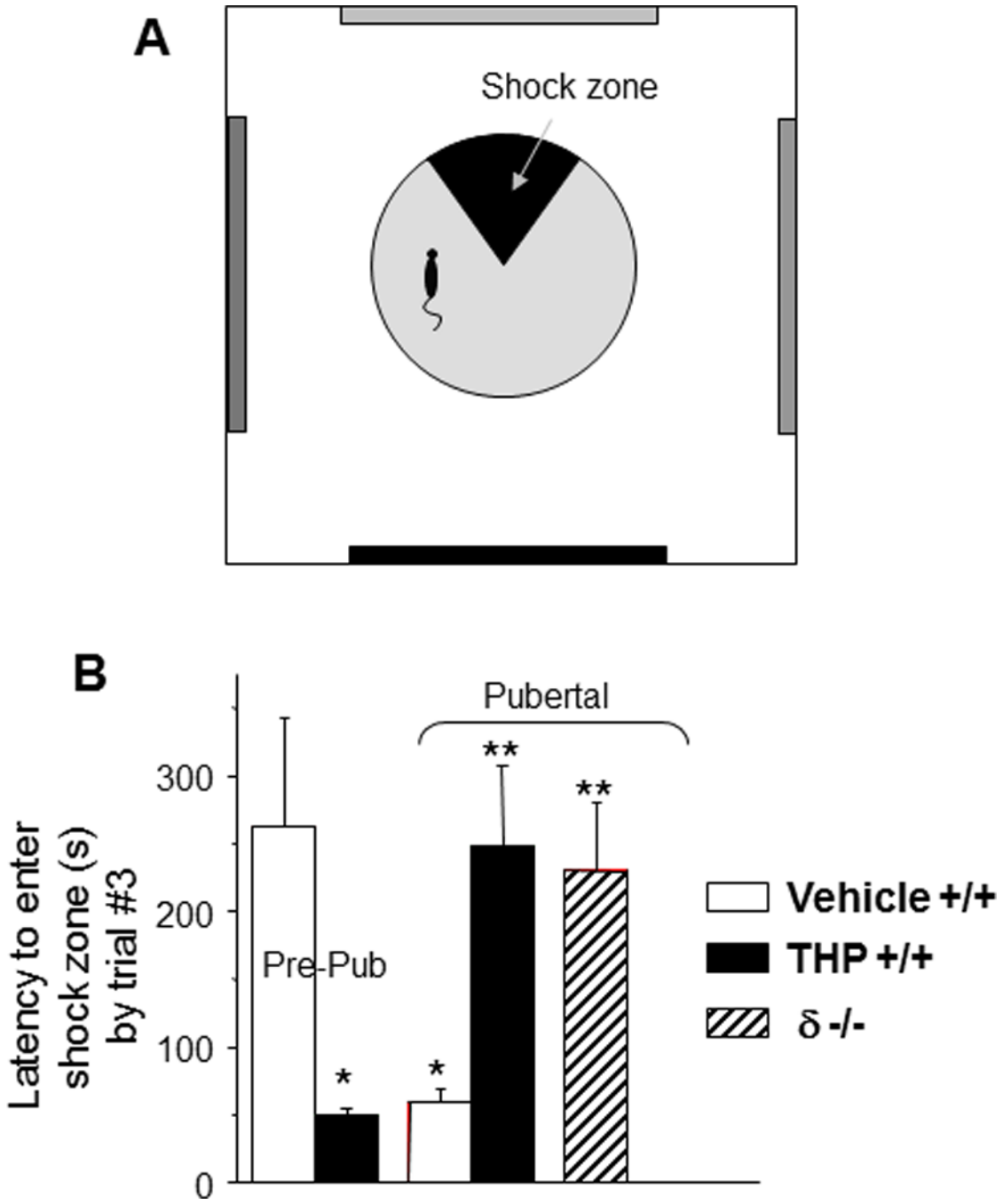

Figure 10. Spatial learning is attenuated at puberty: reversal by the stress steroid THP and β knock-out

A. Spatial learning platform (shock zone, black sector). The platform rotates, but the shock zone is stationary, requiring the animal to move to actively avoid the shock. B. Latency to first entry of the shock zone, a measure of learning. Pre-pub mice attained the longest entry times. (n= 6 – 9 mice/group) *P<0.05 vs. pre-pub.

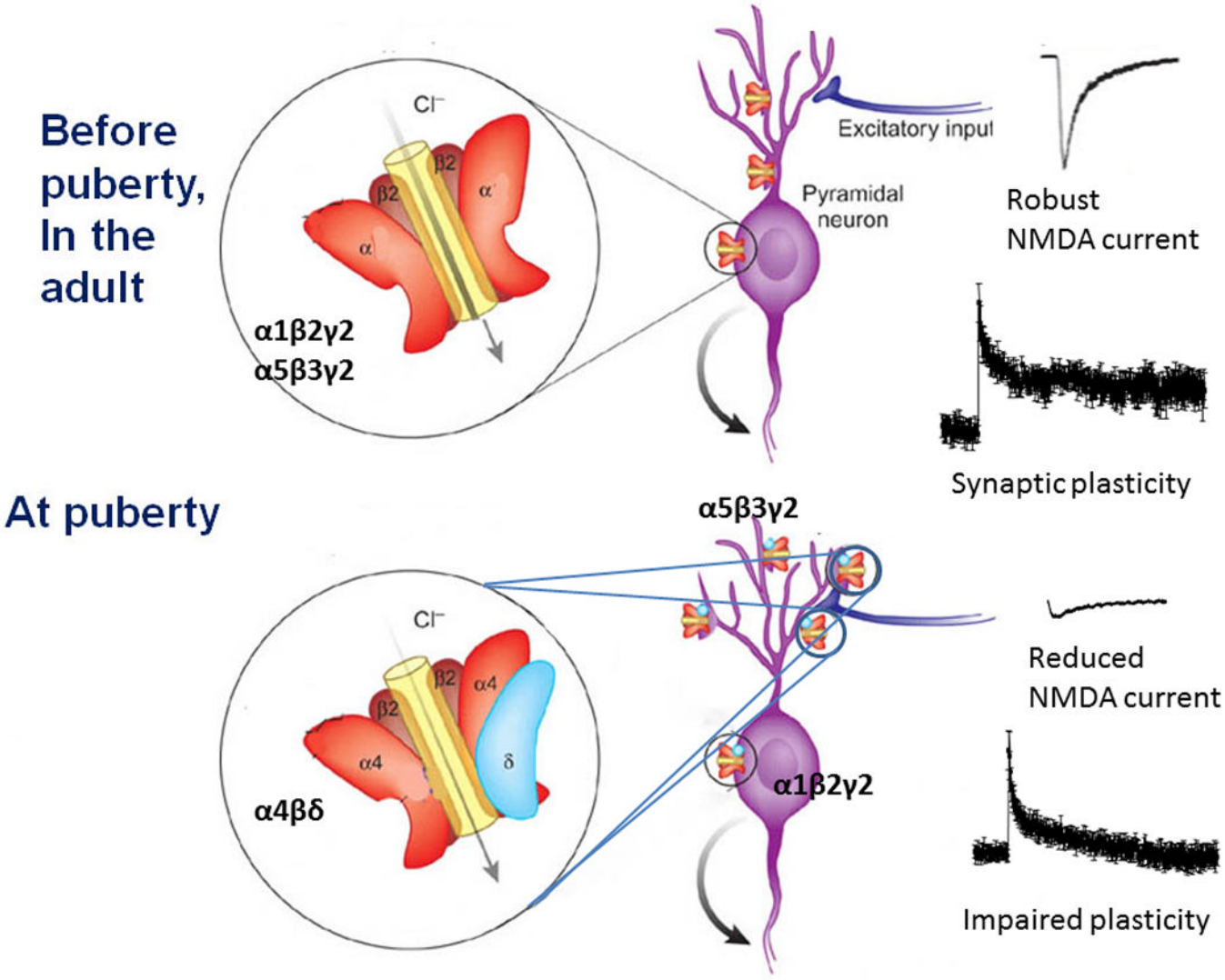

Figure 11. Summary of the effects of α4βδ GABARs on CA1 hippocampal pyramidal cells on plasticity at puberty

Before puberty and in the adult (upper panel) α4βδ GABAR expression is low. Because existing GABARs do not express on dendritic spines, NMDA receptors are easily activated and induction of LTP, a form of synaptic plasticity, is robust. At puberty (lower panel), α4βδ GABARs emerge on the dendritic spines, producing a shunting inhibition, which impairs NMDA receptor activation and synaptic plasticity. Thus, α4βδ GABAR expression at puberty plays a role in limiting the synaptic plasticity that underlies certain cognitive processes. (Revised and used with permission (McCarthy, 2007).)