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Sex differences in anxiety, sensorimotor gating and expression of the $\alpha 4$ subunit of the GABA_A receptor in the amygdala after progesterone withdrawal

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Abstract

In a progesterone withdrawal (PWD) model of premenstrual anxiety, we have previously demonstrated that increased hippocampal expression of the $\alpha 4$ subunit of the GABA_A receptor (GABA_A-R) is closely associated with higher anxiety levels in the elevated plus maze. However, several studies indicate that sex differences in regulation of the GABA_A-R in specific brain regions may be an important factor in the observed gender differences in mood disorders. Thus, we investigated possible sex differences in GABA_A-R subunit expression and anxiety during PWD. To this end, we utilized the acoustic startle response (ASR) to assess anxiety levels in male and female rats undergoing PWD as the ASR is also applicable to the assessment of human anxiety responses. We also investigated GABA_A-R $\alpha 4$ subunit expression in the amygdala, as the amygdala directly regulates the primary startle circuit. Female rats exhibited a greater ASR during PWD than controls, indicating higher levels of anxiety and arousal. In contrast, male rats undergoing PWD did not demonstrate an increased ASR. The sex differences in the ASR were paralleled by sex differences in the expression of the GABA_A-R $\alpha 4$ subunit in the amygdala such that $\alpha 4$ subunit expression was up-regulated in females during PWD whereas $\alpha 4$ levels in males undergoing PWD were not altered relative to controls. These findings might have implications regarding gender differences in human mood disorders and the aetiology of premenstrual anxiety.

Keywords

acoustic startle response; allopregnanolone; amygdala; anxiety; GABA_A receptor; neurosteroid; premenstrual syndrome; progesterone withdrawal; sex differences; $\alpha 4$ subunit

Introduction

The regulation of anxiety is integrally associated with the function of the GABA_A receptor (GABA_A-R; Sanders & Shekhar, 1995; Sundström *et al.*, 1998; Crestani *et al.*, 1999). In fact, unique GABA_A-R isoforms in specific brain regions may modulate anxiety differentially (Crestani *et al.*, 1999; Menard & Treit, 1999). Furthermore, endogenous modulators of the GABA_A-R, such as the neurosteroid metabolites of progesterone, regulate GABA_A-R subunit expression and function (Smith *et al.*, 1998a, b; Gulinello *et al.*, 2001, 2002). There are several lines of evidence that indicate strongly that endogenous fluctuations of neurosteroids, such as those that occur during the human menstrual cycle and the rodent oestrous cycle, could play a role in mood and anxiety disorders.

Acute administration of neurosteroids is anxiolytic (Akwa *et al.*, 1999; Bitran *et al.*, 1995), consistent with their role as positive modulators of GABA_A-R-gated chloride influx (Majewska *et al.*, 1986). However, in contrast to acute administration, chronic administration of, and withdrawal from, 3 α -5 α -THP increases GABA_A-R α 4 subunit expression, decreases total GABA-gated current and alters the GABA_A-R response to several classes of GABA modulatory drugs (Smith *et al.*, 1998a, b; Gulinello *et al.*, 2002). Chronic exposure to and/or withdrawal from chronically elevated neurosteroid levels is also correlated with hyperexcitability, anxiety and cognitive deficits (Smith *et al.*, 1998a, b; Ladurelle *et al.*, 2000; Reilly *et al.*, 2000; Gulinello *et al.*, 2002; Johansson *et al.*, 2002). This is consistent with the withdrawal effects of other GABA modulators, such as benzodiazepines, barbiturates and alcohol (Rassnick *et al.*, 1992; Suzuki *et al.*, 1992; Rasmussen *et al.*, 1993; Follesa *et al.*, 2001;).

Many of the effects of progesterone withdrawal (PWD) are observed in both male and female rats (Gulinello *et al.*, 2002). These data are consistent with the body of evidence suggesting that neurosteroids are relevant modulators of behaviour in males in addition to females (Uzunova *et al.*, 1998; Ladurelle *et al.*, 2000). However, other groups have demonstrated gender differences in GABA_A-R function (Wilson & Biscardi, 1997), in the anxiolytic effects of 3 α -5 α -THP (Wilson & Biscardi, 1997; Zimmerberg *et al.*, 1999) and in animal models of anxiety and mood disorders (Johnston & File, 1991; Lehmann *et al.*, 1999; Zimmerberg *et al.*, 1999). Although apparent gender differences in anxiety behaviours could be dependent on the paradigm utilized to assess anxiety (Johnston & File, 1991), the majority of these data corroborate the reports of gender differences in the prevalence and severity of human mood disorders (Shear, 1997; Pigott, 1999). We therefore further investigated gender-dependent responses of anxiety and GABA_A-R subunit expression in the PWD model.

To this end, we utilized the acoustic startle reflex to assess anxiety levels after PWD in male and female rats. The startle reflex is a whole body response to auditory stimuli which has a similar circuitry and pharmacology in humans as it does in animals (Davis, 1980; Lang *et al.*, 1990; Davis *et al.*, 1994; Koch, 1999; Schachinger *et al.*, 1999). Altered acoustic startle responses (ASR) have been demonstrated in anxiety and depressive disorders in humans (Morgan *et al.*, 1996; Allen *et al.*, 1999; Medina *et al.*, 2001) and in animal models of these disorders (Davis *et al.*, 1997; Schwegler *et al.*, 1997; Stohr *et al.*, 1999; Plappert & Pilz, 2002). Furthermore, the ASR is relevant to PWD as chronic exposure and withdrawal from other GABA_A-R modulators (such as alcohol or benzodiazepines) also increases the ASR (Rassnick *et al.*, 1992; Rasmussen *et al.*, 1993; Krystal *et al.*, 1997; Ponomarev & Crabbe, 1999) and can alter the pharmacological effects of GABA_A-R modulators (Davis & Gallager, 1988).

Sensorimotor gating was also assessed by measuring prepulse inhibition (PPI) of the startle response. In this protocol, the magnitude of the response to a target startle stimulus is reduced by the prior presentation of a low-intensity, nonstartling stimulus (Ison *et al.*, 1973). The PPI component of the ASR is thought to reflect an individual's ability to screen or 'gate' sensory stimuli.

Both the magnitude of the ASR and sensorimotor gating of the ASR are subject to regulation by GABA_A-R in several relevant brain regions, notably, the amygdala (Davis, 1980; Davis *et al.*, 1994; Fendt *et al.*, 2000). We also therefore determined GABA_A-R subunit immunoreactivity in the amygdala after PWD.

Materials and methods

Animals

Male and female Long-Evans rats (Charles River) were housed in single gender pairs under a 14 h light : 10 h dark cycle (lights on at 0700 h) with food and water available *ad libitum*. All animals were tested during the light portion of the circadian cycle between 09.00 h and 14.00 h. In female rats, the stage of the oestrous cycle was determined by microscopic examination of the vaginal lavage and by measures of vaginal impedance, as described previously (Gulinello *et al.*, 2002), throughout one entire cycle before testing. Male rats were handled for the same amount of time. Only females in dioestrus were used as subjects. Following the appropriate treatment (see below), animals were either tested behaviourally or were killed by decapitation, the amygdala removed and frozen on dry ice for isolation of plasma membrane fractions and subsequent Western blot analysis. Both female and male rats weighed 250 g (females $\approx$ 70–80 days old; males $\approx$ 60–70 days old) at the time of testing. All protocols were approved by the institutional Animal Care and Use Committee.

Hormone administration

Progesterone was administered rather than $3\alpha,5\alpha$ -THP because it is known that elevated circulating levels of progesterone, such as those found during the oestrous (or menstrual) cycle or after stress (Purdy *et al.*, 1991; Frye *et al.*, 2000; Girdler *et al.*, 2001) are readily converted to $3\alpha,5\alpha$ -THP in the brain and result in 3α - 5α -THP levels sufficient to potentiate GABAergic inhibition (Bitran *et al.*, 1995) and modulate GABA_A-R subunit expression (Smith *et al.*, 1998a, b). Progesterone implants were made from silicone tubing as described previously and implanted subcutaneously under halothane anaesthesia in the abdominal area of the rat (Smith *et al.*, 1998b) for 21 days. This method has been shown to result in central nervous system levels of 3α - 5α -THP in the high physiological range (6–12 ng/g hippocampal tissue; Moran & Smith, 1998). Control animals were implanted with empty (sham) silicone capsules. Animals were tested 24 h after removal of the implant (progesterone withdrawal).

Acoustic startle

Acoustic startle magnitude was assessed using an S-R Laboratory (San Diego Instruments, San Diego, CA, USA). Rats were placed in a 20 cm $\times$ 32 cm plexiglass cylinder attached to a piezoelectric transducer platform to detect the motion of the rat. Movement of the platform results in a voltage change in the transducer, which was digitized and analysed by the S-R Laboratory program on an attached computer. After a 5 min period of acclimatization to 65 dB background noise, rats were presented with nine consecutive trials of 120 dB sound pulses of 40 ms duration in a habituation trial. Immediately thereafter startle magnitude and threshold were assessed by presentation of broadband noise of varying intensities (0, 90, 110 or 120 dB) presented a total of five times in random order with random time intervals separating each trial. Startle magnitude was defined as an average of responses to each intensity of stimulus and is illustrated in the Results sections either as the maximum or the total integrated startle response. Prepulse inhibition was then assessed by presentation of a low intensity, nonstartling pulse immediately (80 ms) preceding a target 120 dB pulse. Prepulses of 2, 4, 8, and 16 dB above background were presented a total of five times each in random order in addition to five target pulses of 120 dB not preceded by prepulses. Results from PPI trials are illustrated as mean percentage inhibition compared with the mean response to 120 dB target pulses (%PPI). In addition, the absolute startle magnitude (total Integral) to the target pulse following the prepulse is also represented.

Western blot $\alpha 4$ levels were measured in amygdala crude membrane fractions using Western blot procedures explained in detail elsewhere (Smith *et al.*, 1998b). Immunoreactivity of the $\alpha 4$ band (67 kDa) was probed with an antibody developed against a peptide sequence of the

rat $\alpha 4$ subunit (amino acids 517–523) using ECL (enhanced chemiluminescence) detection and quantified using One-Dscan software (Smith *et al.*, 1998a). The results were standardized to a glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 36 kDa) control protein and were then expressed as a ratio of the average optical density of control values (Gulinello *et al.*, 2002).

Materials

Chemicals were obtained from Sigma (St Louis, MO, USA) unless otherwise indicated. The $\alpha 4$ antibody was produced by Sigma Genosys (The Woodlands, TX, USA), and the GAPDH antibody Chemicon (Temecula, CA, USA). ECL supplies were provided by Pierce Biotechnology (Rockford, IL, USA). Silicone tubing and adhesive were obtained from Nalgene Co. (Rochester, NY, USA) and Dow Corning Corp. (Midland, MI), respectively.

Statistical analysis

Differences between groups in Western blots were assessed using an unpaired Student's *t*-test (two-tailed). Data from the startle test and PPI were analysed in a two-way analysis of variance (ANOVA) with weighted means (condition $\times$ sex) followed by *post hoc t*-tests (Bonferroni/Dunnett). Statistical significance for each analysis is indicated in the relevant Results section. Slopes of the startle habituation curves were also analysed following linear regression and are indicated in the Results.

Results

PWD increases acoustic startle response in female but not male rats

There were significant gender differences in anxiety as measured by the acoustic startle magnitude in male control rats compared with female controls, such that female rats startle less than their male counterparts ($P < 0.01$, Fig. 1). In addition, female rats undergoing PWD had a significantly higher ASR compared to sham implanted females (Fig. 1) as assessed by an increased total integrated startle response (Fig. 1A) and by the maximum response (Fig. 1C, $P < 0.01$). In contrast, male rats undergoing PWD had a significantly decreased ASR compared with sham implanted controls at one stimulus intensity (Fig. 1B and D; $P < 0.01$). There were no significant differences in the latency of the startle response in any of the treatment groups (Fig. 1E and F). We have previously demonstrated that locomotor activity is not significantly affected by PWD (Gulinello *et al.*, 2002). Additionally, there were no mean differences in body weight across gender or treatment conditions by MANOVA analysis of startle data [Female-control = 250 g $\pm$ 0.10; Female-PWD = 260 g $\pm$ 0.10; Male-control 262 g $\pm$ 0.11; Male-PWD 273 g $\pm$ 0.10; $F(\text{weight})_{\text{condition} \times \text{sex}} = 0.22$, $P < 0.883$].

Similar anxiogenic effects of PWD in female rats were also evident when an analysis of the response to the first stimulus was performed (Fig. 2). This measure is generally assumed to be free of effects of sensitization and habituation (Plappert & Pilz, 2002). Female rats undergoing PWD demonstrated a roughly twofold increase in peak ASR to the first presentation of a 120 dB stimulus (Fig. 2A; $P < 0.02$). Although males undergoing PWD were not significantly different relative to sham implanted males (Fig. 2B), control males had a higher peak ASR than control females (Fig. 2A and B).

The habituation responses to the first nine consecutive presentations of a 120 dB stimulus were also examined (Fig. 2C and D). The slope of the habituation curve in both PWD females (-33.6 ± 49.35) and control females (-59.27 ± 13.59) was significantly different than 0 ($P < 0.03$ and $P < 0.003$, respectively). Furthermore, the slopes between PWD and controls animals did not significantly differ in females ($P < 0.2$) indicating that habituation occurs in both groups and is not impaired by PWD. In contrast the elevation, or y-intercept, of the slopes was significantly

different between females undergoing PWD (2336 ± 251.8) and controls (1015 ± 76.48 , $P < 0.001$), indicating a higher startle magnitude during PWD throughout the habituation trials.

This pattern was different in males. Male controls had a significantly steeper habituation slope (-236.7 ± 39.89 , $P < 0.001$) than male rats undergoing PWD (-62.15 ± 43.05). Furthermore, male controls had a significantly steeper habituation slope than control females ($P < 0.001$), a characteristic that has been reported previously (Rinaldi & Thompson, 1985; Blaszczyk & Tajchert, 1996; Lehmann *et al.*, 1999).

PWD increases sensorimotor gating in female but not male rats

Despite the fact that female rats undergoing PWD had a larger ASR, sensorimotor gating (as measured by prepulse inhibition) was preserved in these subjects (Fig. 3). Presentation of a nonstartling stimulus (2–16 dB above background) reliably results in a decreased startle response to a target pulse of 120 dB, known as prepulse inhibition (PPI). Female rats undergoing PWD had significantly increased PPI when these data were expressed as a percentage of the total integrated target pulse response ($P < 0.01$, Fig. 3A). In contrast, male rats undergoing PWD did not differ in any measure of sensorimotor gating when compared to controls (Fig. 3). There were no significant differences in percentage PPI between male and female control rats. Female rats undergoing PWD had no significant differences in PPI compared with control rats as a percentage of the maximum response to the target pulse (Fig. 3).

Sex differences in the GABA_A-R $\alpha 4$ subunit expression in the amygdala following PWD

The levels of GABA_A-R $\alpha 4$ subunit in the amygdala increased 2–3-fold after PWD in female rats. In contrast, there were no significant changes in $\alpha 4$ subunit expression in male rat amygdala membranes (Fig. 4). The levels of GAPDH control protein did not differ between treatment conditions. These data differ from our previously published results in the hippocampus, where the $\alpha 4$ subunit is upregulated in the hippocampus of both male and female rats during PWD (Gulinello *et al.*, 2002).

Discussion

These data demonstrate increased, gender-dependent expression of the $\alpha 4$ subunit of the GABA_A-R in the amygdala in association with increased anxiety (measured by increased acoustic startle response) in female rats undergoing PWD. In contrast, male rats exhibit neither increased ASR nor any alteration in $\alpha 4$ subunit expression in the amygdala during PWD. The results from this rodent model of pre-menstrual hormone fluctuations are consistent with the fact that there are corresponding gender differences in the prevalence and severity of mood disorders in humans, which are thought to be related to neuroendocrine factors (Shear, 1997; Pigott, 1999).

The increased anxiety demonstrated in female rats undergoing PWD occurs in close association with increased expression of the $\alpha 4$ subunit of the GABA_A-R in several tasks (Gallo & Smith, 1993; Smith *et al.*, 1998b; Gulinello *et al.*, 2002). During PWD, female rats exhibit increased anxiety in the buried shock-probe paradigm (Gallo & Smith, 1993), and in the elevated plus maze (Smith *et al.*, 1998b; Gulinello *et al.*, 2002) in addition to the increased startle magnitude demonstrated here. Withdrawal from 3 α -5 α -THP after chronic exposure to its parent compound, progesterone, also decreases the sensitivity of the GABA_A-R to several GABA_A-R modulators (Smith *et al.*, 1998a, b; Gulinello *et al.*, 2001, 2002) and decreases total GABA-gated current (Smith *et al.*, 1998a, b). Our recent studies (Sundstrom-Poromaa *et al.*, 2002) also demonstrate that the ASR is uniquely sensitive to modulation by low doses of alcohol in female rats following PWD, an effect produced by increased expression of $\alpha 4\beta\delta$ GABA_A-R

at this time. These phenomena could contribute to relevant behavioural outcomes, such as changes in emotional behaviour, because the degree of sensitivity to GABA_A-R modulators is correlated with the severity of negative symptoms in mood disorders (Sundström *et al.*, 1998).

However, whereas males subjected to the PWD paradigm exhibit increased anxiety in the elevated plus maze (Gulinello *et al.*, 2002) they do not demonstrate increased startle magnitude as do their female counterparts, suggesting that the effects of PWD are regulated in a gender-dependent way in specific brain regions. One possible explanation of these results is that neurotransmitter systems relevant to the modulation of anxiety might be regulated by neurosteroid withdrawal in a sexually dimorphic way in specific brain regions.

There are indeed sexually dimorphic features of limbic brain regions known to regulate anxiety, and these include the amygdala (Reisert *et al.*, 1996; Wilson & Biscardi, 1997; Stefanova, 1998). The primary startle pathway consists of the auditory nerve, the cochlear root neurons, the nucleus of the lateral lemniscus, the pontine nuclei and spinal interneurons and motoneurons (Fendt & Fanselow, 1999; Koch, 1999). This primary circuit is regulated through a direct input from the amygdala (and other regions) (Davis *et al.*, 1982; Rosenet *et al.*, 1991; Fendt & Fanselow, 1999; Koch, 1999). GABA_A-R expression is also regulated in a sex-dependent manner (Stefanova, 1998; Wilson & Biscardi, 1997) which could be an important factor in the regulation of the ASR (Kellogg *et al.*, 1991; Fendt, 1999; Koch, 1999; Fendt *et al.*, 2000; Bast *et al.*, 2001).

The significant sex differences in $\alpha 4$ subunit expression in the amygdala closely parallel sex differences in behaviour following PWD. In fact, although we have previously demonstrated that PWD increases expression of the $\alpha 4$ subunit in the hippocampus of both male and female rats, in the present study we show that PWD increases $\alpha 4$ subunit expression in the amygdala only in female rats. Several groups have noted similar sex differences after withdrawal from other GABA_A-R modulators, such as benzodiazepines, barbiturates and ethanol (Devaud *et al.*, 1999; Suzuki *et al.*, 1992; Pesce *et al.*, 1994). It is interesting to note in this context that alcohol withdrawal, which also increases $\alpha 4$ subunit expression in the hippocampus (Devaud *et al.*, 1997; Mahmoudi *et al.*, 1997), decreases expression of the $\alpha 4$ subunit of the GABA_A-R in the amygdala of male rats (Papadeas *et al.*, 2001).

In this context, it is also noteworthy that males have a higher baseline startle response than females. The increased startle response, in addition to other characteristic sex differences, such as the steeper habituation curve evident in males, which have been previously demonstrated by other groups (Rinaldi & Thompson, 1985; Blaszczyk & Tajchert, 1996; Lehmann *et al.*, 1999), are reversed by chronic exposure to and withdrawal from progesterone in males. There are several variables that might contribute to this pattern of results. It has been suggested elsewhere that the apparently higher startle response in control males could be partially a function of a stronger muscular response in male animals (Blaszczyk & Tajchert, 1996). This hypothesis would be consistent with the lack of sex differences in control animals that we have reported previously in the elevated plus maze (Gulinello *et al.*, 2002).

Alternatively, these sex differences in baseline startle in control animals could be the result of sexually dimorphic reactions to stressors (Akinci & Johnston, 1993; Farabollini *et al.*, 1996; Wood *et al.*, 2001; Figueiredo *et al.*, 2002). It has been demonstrated elsewhere, for example, that chronic testosterone exposure has opposite effects on behaviour and GABA-ergic transmission as exposure to progesterone (Bitran *et al.*, 1993), suggesting that testosterone might also play a role in the manifestation of anxiety. Furthermore, both oestrogen and testosterone regulate the sexually dimorphic response to stressors (Viau & Meaney, 1996; Shors *et al.*, 1999; Wood *et al.*, 2001). The higher oestrogen levels in females could also be

important in the manifestation of anxiety, as oestrogen interacts with progesterone and 3α - 5α -THP to regulate neuronal excitability and anxiety (Becker & Rudick, 1999; Chesler & Juraska, 2000; Cyr *et al.*, 2000; Laconi *et al.*, 2001) and might also have independent effects on both inhibitory and excitatory transmission (Woolley, 1999).

These factors could also be important in the striking differences between male and female rats undergoing PWD. Other groups have also demonstrated that withdrawal from GABA-modulators is more severe in females than males (Pesce *et al.*, 1994). However, it is also possible that male startle responses have been 'feminized' by chronic exposure to progesterone. It has certainly been demonstrated elsewhere that exposure to progesterone in males can induce patterns of behaviour typical of females (Fraile *et al.*, 1988). Although we have previously demonstrated that the PWD syndrome is not replicated by chronic exposure to progesterone in females (Smith *et al.*, 1998b; Gulinello *et al.*, 2001) we can not rule out that there are such effects in males.

Sensorimotor gating is preserved and even augmented in female rats undergoing PWD despite their elevated initial startle response. The increased startle magnitude and the preservation of intact sensorimotor gating in this rodent model of premenstrual dysphoria are consistent with the fact that the primary symptoms of this disorder tend to be affective and mood symptoms rather than other psychiatric deficits that are more closely associated with disrupted gating (Braff *et al.*, 1978; Perry *et al.*, 1999).

Although these data might initially appear paradoxical, it has been demonstrated previously that PPI and startle magnitude can be regulated differentially (Bullock *et al.*, 1997; Stohr *et al.*, 1999) and we suggest that these results might be consistent with alterations in GABA-gated current resulting from increased $\alpha 4$ subunit expression during PWD (Smith *et al.*, 1998a; Smith *et al.*, 1998b). The increased ASR during PWD in females is certainly consistent with the body of literature demonstrating that the ASR is reliably enhanced by GABA_A-R antagonists and is conversely decreased by GABA-ergic anxiolytics (Davis & Gallager, 1988; Hijzen & Slangen, 1989; Kellogg *et al.*, 1991; Davis *et al.*, 1994; Davis, 1998; Guscott *et al.*, 2000; Walker & Davis, 2002).

This, of course, does not account for the fact that PPI is preserved during PWD despite the increased ASR, although there are precedents for such responses. Anticipatory anxiety and greater attention to threat cues can increase PPI (Grillon & Davis, 1997). Furthermore, GABA_B receptor mutant mice also show increased behavioural excitability in conjunction with increased PPI as the result of their decreased receptor function (Prosser *et al.*, 2001). However, the literature regarding the pharmacology and mechanisms of the regulation of PPI is not always consistent, and is a function of the specific brain region investigated (Kodsi & Swerdlow, 1995; Bast *et al.*, 2001; Swerdlow *et al.*, 2001). This could be an important factor, as the expression of the atypical $\alpha 4$ subunit is also restricted to specific brain regions (Pirker *et al.*, 2000).

Several groups have demonstrated that that PPI is disrupted by agents that increase GABAergic inhibition, such as benzodiazepines (Depoortere *et al.*, 1997; Schachinger *et al.*, 1999) and high levels of neurosteroids during proestrus (Koch, 1998). Furthermore, blockade of GABA_A-R in the superior colliculus, where there is $\alpha 4$ expression, also increases PPI (Fendt, 1999; Pirker *et al.*, 2000). Conversely, activation of GABA_A-R in regions with strong $\alpha 4$ expression reduces PPI (Kodsi & Swerdlow, 1997). In contrast, blocking GABA_A-R function by picrotoxin in the ventral striato-pallidal pathway reduces PPI. However, $\alpha 4$ subunits are not expressed there substantially, and are unlikely to affect PPI through these pathways (Pirker *et al.*, 2000). Furthermore, whereas GABA_A-R antagonists injected into the amygdala reduce PPI, so do glutamate receptor antagonists (Bakshi *et al.*, 1999; Fendt *et al.*, 2000), which

strongly indicates that PPI is a network property, regulated by specific cell types in specific brain regions, and not a simple function of general excitability (Bakshi & Geyer, 1998; Swerdlow *et al.*, 2001).

In addition, the characteristics of PPI are also regulated by several other major neurotransmitter systems (Fendt *et al.*, 2000, 2001; Braff *et al.*, 2001) which have not been investigated here. It has, for example, been demonstrated previously that both 3 α -5 α -THP and progesterone have neuroleptic properties that are mediated through non-GABA_A-R mechanisms (Maurice *et al.*, 1999; Rupprecht *et al.*, 1999).

In summary, in a rodent model of premenstrual anxiety, we have demonstrated sex differences in expression of the α 4 subunit of the GABA_A-R in the amygdala which occur in association with sex differences in the manifestation of anxiety in a task that is influenced by the amygdala. Further investigation of sex differences in the regulation of GABA_A-R subunit expression has important implications for the underlying aetiology and gender differences in human mood disorders.

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Abbreviations

3 α -5 α -THP	3 α -hydroxy-5 α -pregnan-20-one or allopregnanolone
ASR	acoustic startle response
dB	decibel
ECL	enhanced chemiluminescence
GABA _A -R	GABA _A receptor
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
PPI	prepulse inhibition
PWD	progesterone withdrawal

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Fig. 1.

Progesterone withdrawal results in sex differences in the acoustic startle response. This figure illustrates the sex differences in measure of acoustic startle response (ASR, vertical axis) to varying sound pulses (horizontal axis). (A and C) PWD increases the ASR in female rats. Both the total integrated ASR (A) and the peak ASR (C) is higher in female rats undergoing PWD ($n = 7$ closed circles) than in sham implanted controls ($n = 7$, open circles). Significant differences between PWD and control groups ($P < 0.01$) are indicated by (*), whereas significant sex differences between controls ($P < 0.01$) are indicated by (+) in this and the following graphs. (B and D) PWD decreases the ASR in male rats. Both the total integrated ASR (B) and the peak ASR (D) are lower in male rats undergoing PWD ($n = 7$, closed triangles)

than in sham implanted controls ($n=7$, open triangles). Furthermore, male control rats startle significantly more than control females and males undergoing PWD startle significantly less than control males. (E and F) Latency of the ASR is not affected by PWD. There are no significant differences in the latency of the ASR across treatment conditions or sex.

Fig. 2.

Response to the first stimulus and habituation curves. (A and B) Peak ASR to the first presentation of an acoustic stimulus (120 dB) in female (A) or male (B) rats. Sample sizes are the same as the previous graph. Statistically significant increased ASR in PWD females relative to controls is indicated by (*, $P < 0.05$). Increased ASR in control males relative to control females is indicated by (+, $P < 0.05$). (C and D) Habituation curve of the peak ASR to nine consecutive presentations (trial no, horizontal axis) of a 120 dB stimulus in female (C) or male (D) rats.

Fig. 3.

PWD increases sensorimotor gating in female rats. Sensorimotor gating is indicated as percentage PPI (either percentage total integrated response or percentage peak response, vertical axis) following presentation of increasing decibels of acoustic stimuli (horizontal axis). (A and B) PWD increases percentage PPI in female rats. Sensorimotor gating is increased in female rats undergoing PWD when gating is assessed as a percentage of inhibition relative to the total integrated target pulse (A) or as a percentage of the peak response (B). Sample sizes and significance levels are the same as in Fig. 2. (C and D) PWD does not alter percentage PPI in males. Relative PPI is not affected in male rats undergoing PWD when expressed as percentage integrated response (C) or as percentage peak response (D), nor are there any significant sex differences in percentage PPI.

Fig. 4.

PWD increases the expression of the $\alpha 4$ subunit of the GABA_A-R in the amygdala of female but not male rats. (A) Representative Western blot. This blot illustrates sex differences in expression of the $\alpha 4$ subunit in the amygdala following PWD. Lanes 1 and 2, female control; lanes 3 and 4, female PWD; lanes 5 and 6, male control; lanes 7 and 8, male PWD. $\alpha 4$ Subunit immunoreactivity is indicated by arrows. The levels of the GAPDH control protein (also indicated by arrows) do not change in any treatment condition. (B) Averaged data. Immunoreactivity of the $\alpha 4$ subunit of the GABA_A-R, averaged data. The sample size for each group is indicated by the numbers at the base of each bar and statistical significance (*) is $P < 0.01$.