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# Gender Impacts Behavioral and Neurochemical Adaptations in Ethanol-Dependent Rats

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DEVAUD, L. L., D. B. MATTHEWS AND A. L. MORROW. *Gender impacts behavioral and neurochemical adaptations in ethanol-dependent rats.* PHARMACOL BIOCHEM BEHAV **64**(4) 841–849, 1999.—Previous investigations have found gender differences in the effects of chronic ethanol exposure on ethanol withdrawal behaviors as well as $GABA_A$ receptor gene expression. The present investigation extended these studies with additional behavioral and neurochemical measures of ethanol dependence and withdrawal. No significant gender differences in the elevated plus-maze assessment of ethanol withdrawal anxiety behaviors were found. However, the neuroactive steroid, 3α,5α-THP, increased exploratory behavior in ethanol withdrawn female, but not male, rats. $GABA_A$ receptor binding assays showed potent competition of [$^{35}$S]TBPS binding by 3α,5α-THP. Control females displayed a decreased affinity for 3α,5α-THP compared to control males, as evidenced by a nearly 30% increase in the $IC_{50}$ value. There was no significant effect of ethanol withdrawal on 3α,5α-THP modulation of [$^{35}$S]TBPS binding. However, gender differences were observed in the effects of chronic ethanol exposure on $GABA_A$ receptor subunit peptide levels in the hypothalamus. Female rats had a significant increase in peptide levels for the α2 and α3 but not α4 subunit, whereas male rats displayed a significant increase in α4 and α3 but not α2 subunits compared to pair-fed control levels. Chronic ethanol-induced alterations in gene expression in the hypothalamus did not coincide with previous findings in the cerebral cortex. In particular, male rats showed an increase in α1 subunit peptide levels in the hypothalamus, whereas significant decreases in this subunit have been observed in the cerebral cortex. Both female and male rats showed significant increases in the α3 subunit in the hypothalamus but not the cerebral cortex. Taken together, these studies provide additional support for gender-selective effects of chronic ethanol-elicited adaptations at the molecular level. © 1999 Elsevier Science Inc.

Ethanol dependence  Ethanol withdrawal  Gender  $GABA_A$ receptors  [$^{35}$S]TBPS  3α,5α THP  Neuroactive steroids

ALCOHOL abuse and alcoholism exert a tremendous cost on human health. Nearly 20% of adult males have a problem with alcohol abuse or suffer from alcoholism (18). In contrast, only about 5–6% of adult women are alcoholic or abuse alcohol. Although societal influences are likely to play a significant role in these sex differences in problem alcohol use, it is possible that differences in neurobiological regulation could also be involved. Females exist under a different hormonal milieu than males, and considerable evidence has accumulated suggesting that female gonadal steroids affect neurobiological responses, particularly at the level of $GABA_A$ receptors (20,22,28,32,52,53). As alcohol (ethanol) also alters GABAergic neurotransmission, presumably via actions at $GABA_A$ receptors [see (26) for review], the responses to ethanol mediated at the level of $GABA_A$ receptors may be differentially regulated, depending on gender.

Ethanol and steroid hormone derivatives termed neuroactive steroids (1,2,45) both exert significant effects via interactions with $GABA_A$ receptors. One interesting line of investigation has been the exploration of the influence of $GABA_A$ receptor modulators on the effects of ethanol. Although ethanol withdrawn rats display significant crosstolerance to the anticonvulsant effects of benzodiazepines such as diazepam (15), both rats and mice show marked sensitization to the anticonvulsant effects of the GABAergic neuroactive steroid, allopregnanolone (3α-hydroxy-5α-pregnan-20-one or 3α,5α-

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THP) (11,13,21). Moreover, female rats show greater sensitization than male rats (13). 3α,5α-THP is derived from progesterone, and is present at higher levels in females than males. Brain and plasma levels of 3α,5α-THP have been shown to increase with stress (45,48), and to vary across the estrous cycle in laboratory rodents (9,20) and menstrual cycle in women (25). This suggests that the difference in the degree of sensitization to 3α,5α-THP between ethanol withdrawn male and female rats may be due to the normally higher levels of this neuroactive steroid in females.

Chronic ethanol exposure elicits alterations in $GABA_A$ receptors functional responsiveness [see (10,26) for review]. These altered responses do not appear to be due simply to changes in receptor density (16,33,49). Several alternative mechanisms have been proposed to account for these observed adaptations in $GABA_A$ receptor function, including changes in the subunit assembly of expressed receptors (12,38,43,44) and/or posttranslational modifications (8,27,31). Subunit-selective alterations in $GABA_A$ receptor gene expression have been found following chronic ethanol exposure (12,14,35,36,38,39). As pharmacological properties of $GABA_A$ receptors are conferred by subunit composition (29,34,46,47), these findings suggest that alterations in subunit peptide levels for $GABA_A$ receptors may result in the expression of receptors with an altered subunit composition (and function). Given the behavioral differences between ethanol withdrawn male and female rats, we initiated a series of studies to determine the effects of chronic ethanol consumption on $GABA_A$ receptor subunit peptide levels in female vs. male rats. We found significant gender differences for several $GABA_A$ receptor subunit peptide levels in cerebral cortex (15). The present series of investigations was aimed at discerning whether gender modulates additional behavioral and cellular adaptations associated with ethanol dependence in rats.

## METHOD

Male and female Sprague–Dawley rats (Harlan, Indianapolis, IN) were 150–170 g at initiation of experiments. All experiments were conducted according to Institutional Animal Care and Use Committee approved protocols. Rats were administered ethanol (6–7.5%) in a nutritionally complete liquid diet (ICN Biochemicals, Costa Mesa, CA) for 15 days. Control animals were pair fed the same diet with dextrose substituted isocalorically for ethanol, and water was freely available at all times. Fresh diet was provided daily. Animals were maintained on a 12 L:12 D cycle, with lights on at 0700 h. All behavioral testing and tissue harvesting was done between 0900 and 1200 h. Brain areas were rapidly dissected over ice and stored at −80°C until assay. Tissue from ethanol-dependent animals was used for gene expression studies. For ethanol withdrawal determinations, the ethanol-containing diet was removed 6–8 h before studies were conducted. Daily ethanol consumption averaged approximately 12 g/kg for females and 10 g/kg for males. BEC in ethanol-dependent animals varied from 150–240 mg/dl at the time of sacrifice and were undetectable in ethanol withdrawn animals.

Female rats were group housed for at least 7 days after arrival alongside group-housed male rats before separation into individual housing for liquid diet administration. Vaginal smears were taken daily during the course of ethanol administration to monitor female rat cyclicity. Experimental procedures were scheduled to coincide with the majority of female rats being in estrus. In this series of experiments, 83% of female rats were in estrus ± 1 day at the time of the experiment. All animals within a study (males and females) were used on the same day to maintain equivalent ethanol exposure time.

3α,5α-THP was synthesized by Robert H. Purdy, Ph.D., and purchased from Veterans Medical Research Foundation, San Diego, CA. β-Cyclodextrin (2-hydroxypropyl-β-cyclodextrin) was purchased from Research Biochemicals International, Natick, MA, and was used as the vehicle for 3α,5α-THP. [$^{35}$S]*t*-butylbicyclophosphorothionate (100 Ci/mmol) was purchased from New England Nuclear, Boston, MA.

### *Elevated Plus-Maze Methodology*

Anxiety and locomotion/ataxia associated with ethanol withdrawal was measured on an elevated plus-maze in ethanol-withdrawn animals (6–8 h after removal of the ethanol-containing diet). The maze was elevated approximately 1 m above the floor, and contained four 51 cm-long, 11.5 cm-wide arms arranged at right angles. The closed arms had opaque walls 30 cm high, extending the length of the arm. Fifteen minutes prior to testing, each animal was injected IP with 4 mg/kg 3α,5α-THP or vehicle (β-cyclodextrin) and returned to its home cage. At the time of the test, each animal was placed in the center of the maze facing an open arm and allowed to explore for a 5-min session. During the session, the animal's behavior (e.g., number of arm entries and time spent in each arm per entry) was recorded on a computer. Animals were used once only for any determination.

### *[$^{35}$S]t-Butylbicyclophosphorothionate Binding Assay*

Binding assays were performed in the cerebral cortical or cerebellar homogenates harvested from ethanol-withdrawn animals. P2 pellets were obtained by low-speed centrifugation across a sucrose cushion, followed by high-speed centrifugation of the supernatant. Resulting pellets were frozen at −80°C for at least 24 h, then resuspended and washed three more times. The final pellet was resuspended in 20 vol 50 mM NaKBS (10 mM $KH_2PO_4$, 40 mM $Na_2HPO_4$, and 147 mM NaCl, pH 7.2) for a final concentration of approximately 1 mg protein/ml. GABA (5 μM) was included in some assays. Picrotoxin (100 μM) was used to define nonspecific binding. 3α,5α-THP was suspended in DMSO, diluted to 1%, then added to the reaction for a final DMSO concentration of 0.1%. [$^{35}$S]TBPS (2 nM) was added last to initiate incubation. All determinations were run in triplicate. After 90 min at room temperature, incubation was terminated by rapid vacuum filtration followed by three 4-ml washes with ice-cold buffer. Bound radioactivity was measured by liquid scintillation spectroscopy. Protein was determined using the BCA technique (Pierce, Rockford, IL) and was generally 100 μg/tube. Data were analyzed using Prism2 (GraphPad, Inc., San Francisco, CA).

### *Western Blot Analysis*

Immunoblotting was performed on rat hypothalamic homogenates from ethanol-dependent animals as previously described (14). Tissue was homogenized, and total particulate membrane fractions collected by centrifugation. Samples were loaded on gels in a pair-wise fashion corresponding to the pairings during chronic ethanol administration. Protein (20 μg per well) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in an 8–16% Tris-glycine gel using a MiniCell II apparatus (Novex, San Diego, CA). After separation, proteins were transferred

to PVDF membranes (Immobilon-P, Millipore, Bedford, MA). Blots were incubated with selective antibodies for $GABA_A$ receptor α1 (24), α2 (23), α3 (3), α4 (4), β2/3 (Chemicon, Temecula, CA), or γ2 (46) subunits followed by incubation with the appropriate HRP conjugated secondary antibody. Blots were then exposed to chemiluminesent substrate (Super Signal, Pierce, Rockford, IL), apposed to x-ray film, and quantified by densitometric measurements. All comparisons were made within blots, between pair-fed control and ethanol-dependent samples. All comparisons were made within gender, i.e., pair-fed control females with ethanol-dependent females. Blots were subsequently exposed to a second primary antibody directed against β-actin, to verify equivalent protein loading. β-Actin peptide levels did not change following chronic ethanol administration.

*Statistical Analysis*

For plus-maze measurements, the overall effects of ethanol and treatment with a neurosteroid were determined by ANOVA, with post hoc comparisons by Bonferroni/Dunn analysis. Competition binding assay data were analyzed by Prism2 to generate $IC_{50}$ values for the neurosteroid modulation of [$^{35}$S]TBPS binding. Western blot data were analyzed by the Student's paired *t*-test, with 12 individual pairs compared over two or three determinations per subunit utilizing tissue from three independent chronic ethanol exposure studies.

## RESULTS

To determine if the sensitization to neuroactive steroids during ethanol withdrawal generalized to locomotor or anxiety behavioral measures, we utilized the elevated plus-maze technique. We found a significant gender effect with acute administration of 4 mg/kg 3α,5α-THP (Fig. 1) in animals that had not been exposed to ethanol. Female rats displayed a significant increase in open-arm time as well as open-arm entries following neurosteroid administration (evidence for decreases in anxiety), whereas this concentration of 3α,5α-THP had no effect in male rats, $F(3,29) = 3.74$, $p < 0.05$, by Tukey's post hoc comparisons. Ethanol-naive male and female rats displayed similar percent open-arm times and open-arm entries. When plus-maze behaviors were measured in ethanol-withdrawn male and female rats, 3α,5α-THP administration did not elicit a robust reduction in anxiety. However, there was an effect on general exploratory behaviors. Fig. 2 shows that ethanol withdrawal significantly decreased the number of arm entries and increased the time spent in closed arms for both male and female rats. Acute administration of 4 mg/kg 3α,5α-THP reversed the decreased total-arm entries for ethanol-withdrawn female, but not male, rats, $F(7, 58) = 3.74$, $p < 0.05$, by Tukey's post hoc comparisons.

A series of radioligand binding studies were conducted to investigate whether the differences observed in the effects of neuroactive steroids on ethanol-dependent and withdrawal behaviors could be mediated at the level of the [$^{35}$S]TBPS recognition site on $GABA_A$ receptors. [$^{35}$S]TBPS has high affinity and selectivity for a site in the channel of $GABA_A$ receptors. The neuroactive steroid, 3α,5α-THP, inhibited [$^{35}$S]TBPS binding in a dose-dependent manner (Fig. 3). However, there were no differences in 3α,5α-THP competition for [$^{35}$S]TBPS binding between control and ethanol-withdrawn cerebral cortical homogenates. $IC_{50}$ values were 211 ± 16 nM for control and 200 ± 26 nM for ethanol-withdrawn female rats. $IC_{50}$ values were 163 ± 20 nM for control and 180 ± 32 nM for ethanol-withdrawn male rats. This was a 29% decrease in affinity

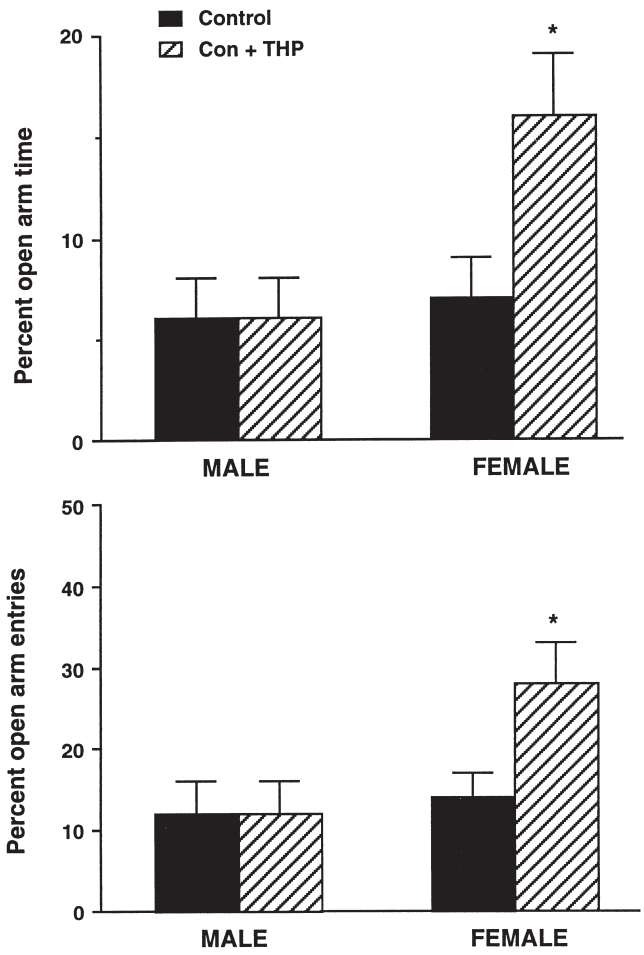


FIG. 1. A single, acute administration of the neuroactive steroid, 3α,5α-THP (4 mg/kg) had significant anxiolytic activity in female but not male rats. Results are presented as percent of time spent in the open arms and percent of entries into the open arms compared to total time and entries over the 5-min session. Values presented are mean ± SE. *$p < 0.05$ compared to control. $n$ = 7–8 rats per gender per treatment group.

of 3α,5α-THP for [$^{35}$S]TBPS binding sites in control female compared to male rats. In ethanol-withdrawn animals, this difference in affinity for [$^{35}$S]TBPS binding was reduced to 11%.

An additional series of experiments were conducted in well-washed membrane homogenates without the addition of GABA (to remove the effect of GABA on [$^{35}$S]TBPS binding). These studies showed a biphasic response for modulation of [$^{35}$S]TBPS binding by 3α,5α-THP (Fig. 4). The enhancement of [$^{35}$S]TBPS binding by 3α,α-THP (1 nm-600 nM) was reduced in cerebral cortices from ethanol-withdrawn male, but not female, rats.

In the cerebellum, 3α,5α-THP competed for [$^{35}$S]TBPS binding sites in the presence of 5 μM GABA with $IC_{50}$ values of 157 ± 2 nM vs. 139 ± 4 nM for control and ethanol-withdrawn female rats, respectively (Fig. 5). Similar $IC_{50}$ values were obtained in male rats, 121 ± 2 nM vs. 153 ± 5 nM for pair-fed control and ethanol-withdrawn animals. As in the cortex, there was a 29% decrease in the affinity of 3α,5α-THP for [$^{35}$S]TBPS binding sites between control female and male rats.

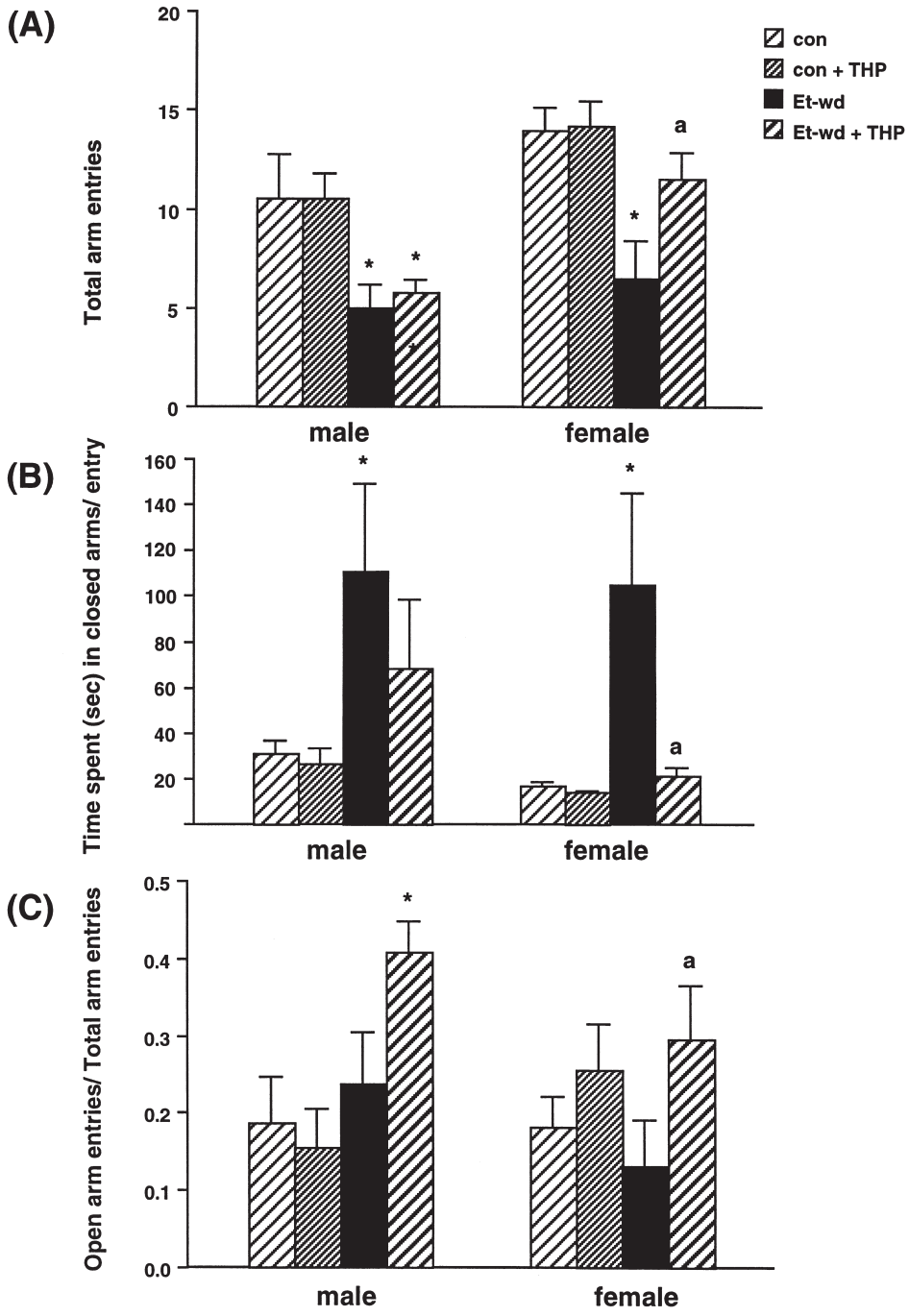


FIG. 2. Ethanol withdrawal reduced general activity (as measured by total arm entries) in both male and female rats. It also significantly increased the time spent in closed arms per entry for both sexes. Acute administration of 3α,5α-THP (4 mg/kg) increased open-arm/total-arm entries in both male and female ethanol withdrawal rats as well as increasing, whereas there was only an effect of 3α,5α-THP in increasing total arm entries in ethanol withdrawn female rats. Values are presented as mean ± SE. $^{*}p < 0.05$ compared to control; $^{a}p < 0.05$ compared to ethanol withdrawn. $n$ = 8–10 animals per gender per treatment group.

An important avenue of research into cellular mechanisms involved in the adaptations associated with ethanol dependence and withdrawal include using molecular approaches to measure $GABA_A$ receptor structure and function. In the present study, we assayed the effects of ethanol dependence in hypothalamic tissue from ethanol-dependent male and female rats. As shown in Fig. 6., there was a significant increase in α1 subunit expression (26 ± 9%) in ethanol-dependent male but not female rats. Conversely, there was a significant increase in α2 subunit levels (26 ± 6%) in ethanol-dependent

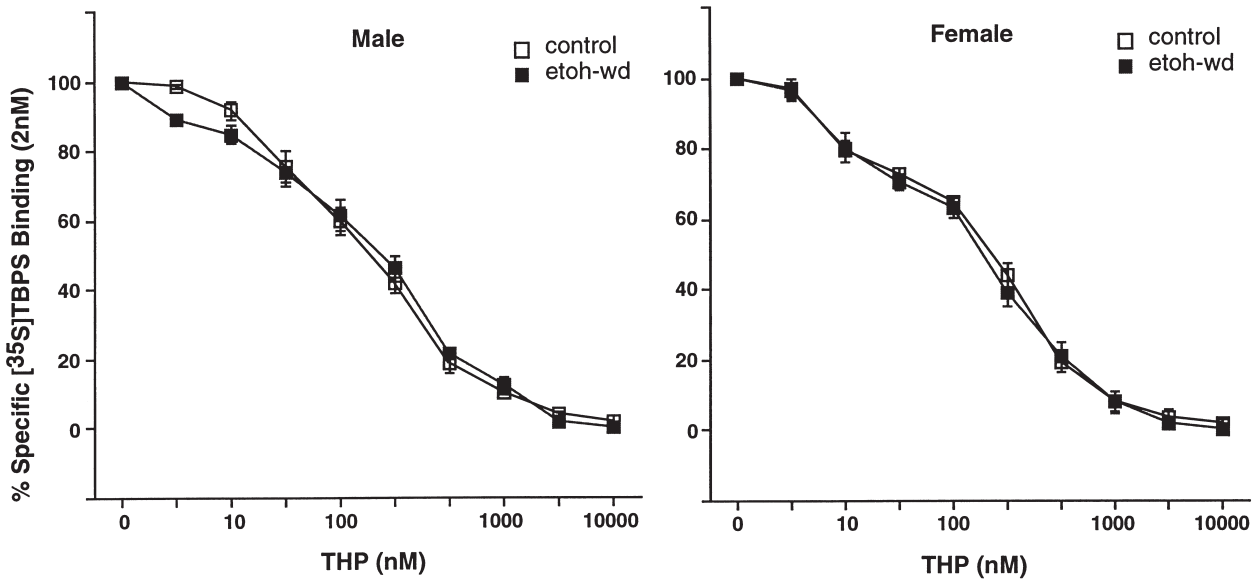


FIG. 3. 3α,5α-THP inhibited [$^{35}$S]TBPS binding to cerebral cortical membranes in a dose-dependent manner. All assays were conducted in the presence of 5 μM GABA. There were no effects of ethanol withdrawal on 3α,5α-THP modulation of binding, although control female rats showed a reduced affinity for 3α,5α-THP inhibition of [$^{35}$S]TBPS binding compared to males. Data presented is a summary (mean ± SE) of four independent experiments run with triplicate determinations in each assay using pooled tissue from two animals per gender per group.

female but not male rats. Levels for the α3 subunit significantly increased in ethanol-dependent animals compared to control levels (44 ± 10% for females and 38 ± 12% for males). The α4 subunit significantly increased (48 ± 15%) in ethanol-dependent male but not female rats. Levels for β2/3 and γ2 subunits were not altered in either sex in the hypothalamus following chronic ethanol exposure. Figure 7 is a comparison of the effects of ethanol dependence on peptide expression for $GABA_A$ receptor subunits in female and male rat cerebral cortex and hypothalamus.

## DISCUSSION

Seizure threshold analysis is a sensitive measure of seizure susceptibility. Small, but significant differences in basal seizure susceptibility between female and male rats have been reported (20,32,42,52). Differential modulation of $GABA_A$ receptor activity by neurosteroids and across the estrous cycle in female rats has also been observed (20,54). Although we found small differences in basal seizure thresholds between male and female rats, we found larger differences in their response to the anticonvulsant effect of the neuroactive steroid,

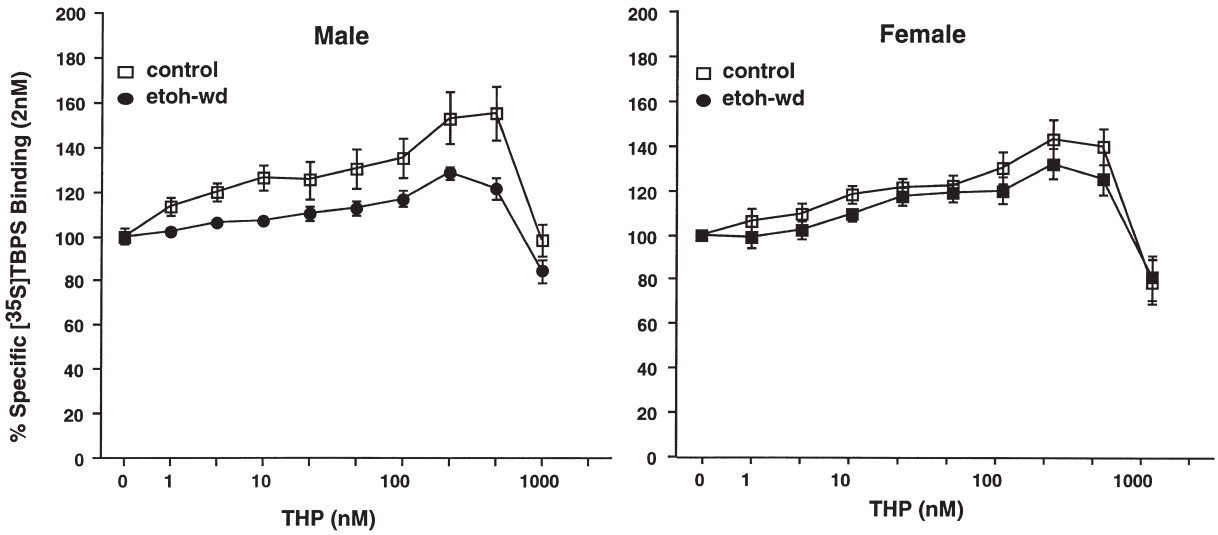


FIG. 4. Cerebral cortical homogenates from ethanol withdrawn male rats showed a reduced enhancement of [$^{35}$S]TBPS binding by 3α,5α-THP in well-washed membranes. This effect was not observed in cerebral cortical homogenates from ethanol withdrawn female rats. Data presented is a summary (mean ± SE) of four independent experiments run with triplicate determinations in each assay using pooled tissue from two animals per gender per group.

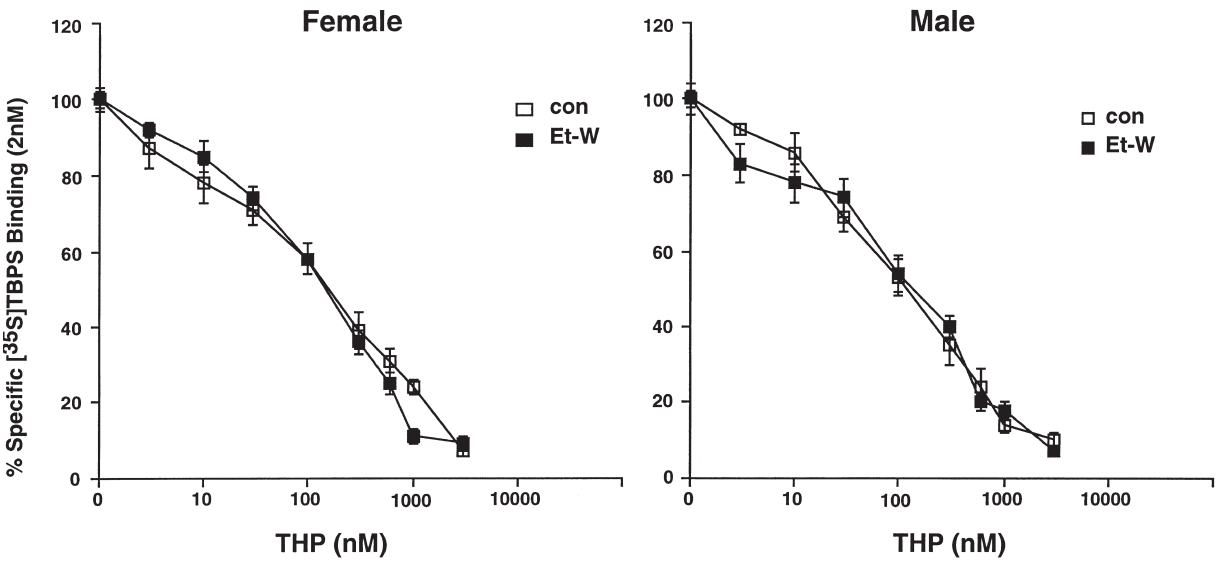


FIG. 5. 3α,5α-THP inhibited [$^{35}$S]TBPS binding to cerebellar membranes in a dose-dependent manner. All assays were conducted in the presence of 5 μM GABA. There were no effects of ethanol withdrawal on 3α,5α-THP modulation of binding, although control female rats showed a reduced affinity for 3α,5α-THP inhibition of [$^{35}$S]TBPS binding compared to males similar to what was observed in cerebral cortex. Data presented is a summary (mean ± SE) of three independent experiments run with triplicate determinations in each assay with tissue from individual animals per gender per treatment group.

3α,5α-THP during ethanol withdrawal (13,15). This sensitization to the anticonvulsant effects of neuroactive steroids in ethanol-withdrawn rats cannot be explained by increases in endogenous levels of 3α,α-THP (30). In an attempt to study whether the sensitization to neuroactive steroids during ethanol withdrawal generalized to other behavioral measures, we employed the elevated plus-maze technique to assay anxiolytic and locomotor effects of 3α,5α-THP. Neurosteroids such as 3α,5α-THP have been shown to possess potent anxiolytic activity in several behavioral paradigms (5–7, 51). The elevated plus-maze is an approach commonly used to study the effects of ethanol [see (17) for review]. We did find some

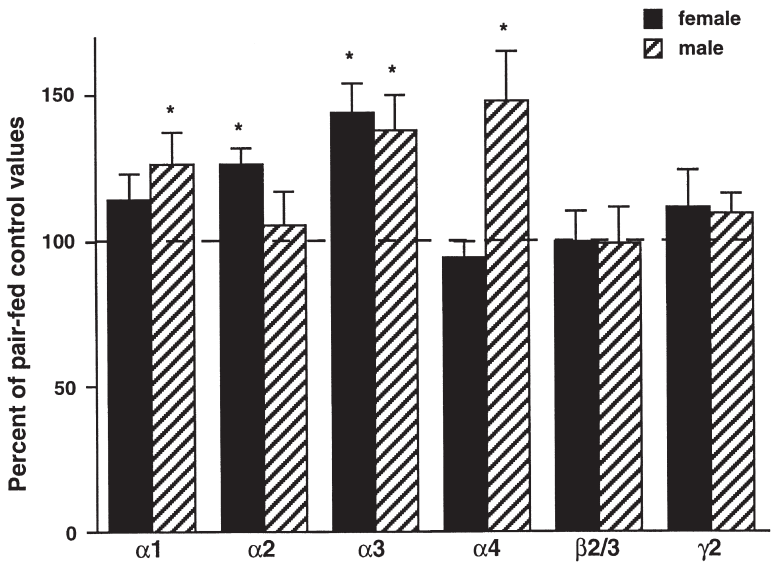


FIG. 6. Western Blot analysis of the effects of ethanol dependence on hypothalamic $GABA_A$ receptor subunit expression showed significant gender differences. Findings are presented as percent of pair-fed determinations. Data were collected from duplicate determinations of 10–12 pairs (pair-fed control and ethanol dependent) per gender over three independent experiments. *$p < 0.05$ compared to control values.

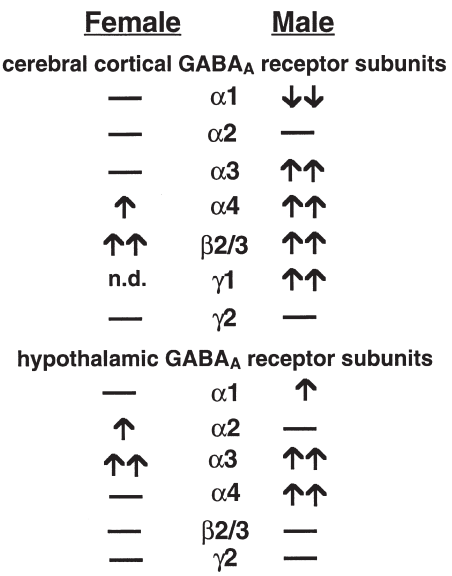


FIG. 7. Ethanol dependence elicits subunit- and gender-selective alterations in $GABA_A$ receptor peptide levels that are also regionally specific. The straight line indicates there was no difference in peptide levels between ethanol dependent and pair-fed control animals; a single arrow denotes changes of 15–25%, a double arrow denotes changes of >26%. This summary is compiled from data obtained over four independent chronic ethanol experiments, and includes measurements from 12–16 pairs of animals per subunit and brain area. $*p < 0.05$.

evidence for increased anxiety during ethanol withdrawal (a significant increase in time spent in closed arms per entry), with a significant reversal of this measure in ethanol-withdrawn female, but not male rats. We did not find a significant increase in open-arm time for either control or ethanol-withdrawn animals administered a single, low dose of 3α,5α-THP, suggesting there was not an anxiolytic effect of 3α,5α-THP in this investigation. However, there was a gender-selective increase in locomotor behavior for ethanol-withdrawn female, but not male, rats following acute administration of 3α,5α-THP (determined by total arm entries). It is not always easy to get definitive results for ethanol-withdrawn rats with the plus-maze. In the study presented here, we found that neither male nor female rats showed decreases in open-arm/total arm entries during ethanol withdrawal. This may be a "floor" effect due to our experimental setup. Control animals had a very limited amount of open-arm entries or open-arm time. It is likely that a higher dose of 3α,5α-THP would detect stronger anxiolytic activity during ethanol withdrawal in rats, whereas the present findings suggest a separation of locomotor from anxiolytic responses to the neuroactive steroid. This assessment of ethanol withdrawal supports the idea that ethanol dependence and withdrawal behaviors are complex.

At a recent satellite symposia to the International Behavioral Neuroscience Society, Dr. Sandra File presented data showing that various behavioral measures (including plus-maze) are differentially indicative of anxiety or locomotion in males compared to females. She presented a complex factorial analysis of data collated from a large number of studies suggesting that in males, open-arm entries is indicative of anxiety, whereas in females, motor activity is the biggest contributor to anxiety behaviors (19). Regardless of the outcomes of this complex analysis, it does suggest that it may be difficult to precisely interpret plus-maze data, especially when considering gender.

Next, the present investigation determined the effects of ethanol withdrawal on modulation of $GABA_A$ receptor binding by [$^{35}$S]*t*-butylbicyclophosphorothionate. Ethanol withdrawal did not alter 3α,5α-THP competition for [$^{35}$S]TBPS binding to cerebral cortical or cerebellar membranes in the presence of a physiologically relevant concentration of GABA. However, we did observe a 29% reduction in the affinity of 3α,5α-THP competition for [$^{35}$S]TBPS binding in females compared to males in both the cerebellum and cerebral cortex. The ability of 3α,5α-THP to modulate [$^{35}$S]TBPS binding in female rats has been shown to vary with the stage of estrus (22). The membrane preparation (i.e., the presence or removal of endogenous substances, including GABA and neuroactive steroids) was found to have a significant effect on the affinity of 3α,5α-THP in that study. In our measurements of 3α,5α-THP modulation of [$^{35}$S]TBPS binding, all tissues were prepared in the identical manner, with male and female samples run in the same assay. This well-washed preparation should have removed any endogenous neuroactive steroids. Therefore, the difference in affinity of 3α,5α-THP modulation of [$^{35}$S]TBPS binding between male and female rats is likely due to differential sensitivity of $GABA_A$ receptors. It suggests that there may be some inherent differences in $GABA_A$ receptor regulation between male and female rats.

We found that 3α,5α-THP modulation of [$^{35}$S]TBPS binding decreased in ethanol-withdrawn male, but not female, cerebral cortical tissue when membranes were well washed to remove all endogenous GABA, and GABA was not added back to the assay. This result agrees with a previous report investigating the effects of chronic ethanol administration on 3α,5α-THP modulation of $GABA_A$ receptor binding by several ligands (37). Recent reports have generated similar findings on the effects of 3α,5α-THP modulation of [$^{35}$S]TBPS binding in well-washed cerebellar and cerebral cortical membranes in male rats (37,50). Both these brain areas appear important in mediation of the effects of chronic ethanol exposure and withdrawal. It is interesting to note that receptor responses to neuroactive steroid modulation are similar in both brain areas, even though affinity for 3α,5α-THP competition of [$^{35}$S]TBPS binding varies by gender. The decreased modulation by 3α,5α-THP of [$^{35}$S]TBPS binding in well-washed membranes from ethanol withdrawn male, but not female, rats may play a role in the enhanced behavioral response to 3α,5α-THP observed in female rats during ethanol withdrawal.

These present findings, taken together with previous evidence, suggest that the development of ethanol dependence involves adaptations in $GABA_A$ receptors. However, the precise mechanisms underlying these adaptations have not been elucidated. Alterations in $GABA_A$ receptor responses associated with ethanol dependence and withdrawal could involve several mechanisms, including alterations in subunit assembly or alterations in postranslational modifications. Several laboratories have generated intriguing data, suggesting both mechanisms may play a role in adaptations associated with chronic ethanol exposure [see (8,26) for review]. A number of previous investigations have found selective alterations in gene expression for $GABA_A$ receptors that vary by brain region (14,38–40,44). We previously found gender-selective effects of ethanol dependence on $GABA_A$ receptor subunit peptide expression in cerebral cortex (15). In the present study, we found gender-selective effects of ethanol dependence on $GABA_A$ receptor subunit peptide expression in hy-

pothalamus. The hypothalamus is an important site for the effects of ethanol, as it is a key player in responding to stress. Furthermore, the hypothalamus appears to be a critical site for steroidal hormone modulation of neuronal activity in both males and females. In addition, there is extensive colocalization of steroid hormone receptors and GABAergic neurons in discrete hypothalamic nuclei (28). This suggests that the differential gender effects of ethanol dependence on $GABA_A$ receptor subunit peptide expression in hypothalamus may have important physiological consequences.

The selective alterations in subunit expression observed in the hypothalamus following chronic ethanol exposure varied from previous findings in the cerebral cortex (15). Notably, the consistent decrease in the $GABA_A$ receptor α1 subunit expression observed in the cerebral cortex from ethanol-dependent male rats was not seen in the hypothalamus. Rather, there was a significant increase in the α1 subunit peptide expression. Alterations in additional $GABA_A$ receptor subunit peptide levels were found to vary by gender and brain region as well. This suggests that there are regionally specific effects of ethanol dependence that may prove to have physiological relevance. The differential effects by brain region likely reflect the different role of various sites in brain. In the hypothalamus, there is a higher level of α2- or α3-containing $GABA_A$ receptors than in most other parts of brain (41,55). $GABA_A$ receptors comprised of α2 or α3 subunits, rather than α1, are classified as type II $GABA_A$ receptors, and display a significantly different pharmacological response to benzodiazepines than type I (α1-containing) receptors (46). The hypothalamus is an integral component of the hypothalamic–pituitary–adrenal (HPA) axis, and plays a role in mediating the stress response. Increased levels of α2 and α3 subunits may indicate an increased density of type II $GABA_A$ receptors in the hypothalamus, which would enhance GABAergic regulation of hypothalamic function. This question remains to be addressed.

The studies presented here and in previous reports show gender-selective effects of ethanol dependence on $GABA_A$ receptor gene expression that varies by brain region. These data have all be collected by Western Blot analysis using within-blot comparisons between treatment groups. A detailed analysis of innate gender differences in peptide levels within regions is an important question that remains to be addressed. If there are basal differences in receptor peptide levels between the sexes, this would suggest that there are gender differences in the normal expression of populations of $GABA_A$ receptors, and would further support the idea that there are significant, inherent gender differences in neurophysiology beyond regulation of reproductive behaviors.

Taken together, findings from the present investigation provide tantalizing evidence suggesting that gender influences neuroadaptations associated with ethanol dependence. These studies also point to the complex regulation of $GABA_A$ receptors that is influenced by the hormonal environment. Evidence for gender-selective effects of ethanol dependence could prove clinically important in the treatment of alcoholism or alcohol withdrawal in females as well as males.

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