JCI The Journal of Clinical Investigation

Gender differences in ethanol preference and ingestion in rats. The role of the gonadal steroid environment.

O F Almeida, … , M H Darwish, V K Patchev

J Clin Invest. 1998;101(12):2677-2685. https://doi.org/10.1172/JCI1198.

Research Article

An ethanol oral self administration paradigm showed the existence of gender differences in alcohol preference in rats: whereas males and females initiated alcohol drinking at similar rates, females maintained their preference for ethanol over a longer duration. Neonatal estrogenization of females, which effectively confers a male phenotype on a genetically female brain, resulted in patterns of drinking that were similar to those displayed by intact male rats, indicating that gender differences in alcohol drinking patterns may be, at least partially, accounted for by sexual differentiation of the brain. To test whether gonadal steroids also exert activational effects on ethanol-seeking behavior, we also examined the effects of gonadectomy alone, or in combination with gonadal steroid replacement therapy. Castration did not significantly alter ethanol consumption in males, although treatment of castrated rats with dihydrotestosterone resulted in a significant inhibition of this parameter. As compared with the situation in intact female rats, ethanol ingestion was significantly reduced in ovariectomized female rats receiving estradiol (E2) and in ovariectomized female rats receiving combined E2 and progesterone replacement therapy. However, neither ovariectomy nor progesterone replacement in ovariectomized rats resulted in ethanol drinking patterns that were different compared to those observed in intact female controls. Thus, dihydrotestosterone and E2, respectively, appear to exert modulatory influences on the male and female rats' preference for ethanol, but further investigations […]

Find the latest version:

https://jci.me/1198/pdf

Gender Differences in Ethanol Preference and Ingestion in Rats

The Role of the Gonadal Steroid Environment

O.F.X. Almeida, M. Shoaib, J. Deicke, D. Fischer, M.H. Darwish, and V.K. Patchev

Neuroadaptations Group, Department of Neuroendocrinology, Max Planck Institute of Psychiatry, D-80804 Munich, Germany

Abstract

An ethanol oral self administration paradigm showed the existence of gender differences in alcohol preference in rats: whereas males and females initiated alcohol drinking at similar rates, females maintained their preference for ethanol over a longer duration. Neonatal estrogenization of females, which effectively confers a male phenotype on a genetically female brain, resulted in patterns of drinking that were similar to those displayed by intact male rats, indicating that gender differences in alcohol drinking patterns may be, at least partially, accounted for by sexual differentiation of the brain. To test whether gonadal steroids also exert activational effects on ethanol-seeking behavior, we also examined the effects of gonadectomy alone, or in combination with gonadal steroid replacement therapy. Castration did not significantly alter ethanol consumption in males, although treatment of castrated rats with dihydrotestosterone resulted in a significant inhibition of this parameter. As compared with the situation in intact female rats, ethanol ingestion was significantly reduced in ovariectomized female rats receiving estradiol (E_2) and in ovariectomized female rats receiving combined E_2 and progesterone replacement therapy. However, neither ovariectomy nor progesterone replacement in ovariectomized rats resulted in ethanol drinking patterns that were different compared to those observed in intact female controls. Thus, dihydrotestosterone and E_2, respectively, appear to exert modulatory influences on the male and female rats' preference for ethanol, but further investigations are necessary to determine to what extent these effects result from activational actions on the brain. (*J. Clin. Invest.* 1998. 101:2677–2685.) Key words: alcohol • sexual differentiation of the brain • gender • addiction

Introduction

The need for a better understanding of the biological mechanisms underlying sex differences in ethanol consumption is being increasingly recognized. For example, there has been a dramatic rise in the estimated prevalence of alcoholism among women living in North America over the last three decades. Whereas the male/female ratio for alcoholism was estimated at 6:1 in the 1960s (1), this ratio was estimated at about 3–4:1 (2) in a recent United States epidemiological survey. Although a general population survey in the United States suggested that the percentage of women who drink declined between 1981 and 1991, one of its findings was that the frequency of alcohol intoxication among younger women was rising (3). The extent to which such trends merely reflect changes in social attitudes (4, 5), or the methods used for collecting epidemiological data, remains obscure. Nevertheless, it is interesting that Dawson and Archer (6) identified the female gender as a risk factor for developing alcohol dependence despite the fact that the daily average ethanol intake by men is about double that of women after adjusting for body weight and body water.

Gender-specific patterns of ethanol ingestion in rodents were not recorded in the older literature (for review see reference 7). However, Eriksson (8), and Eriksson and Pikkarainen (9) reported greater ethanol consumption in female rats (ALKO strain) and mice (C57BL strain), respectively. Juarez et al. (10) reported that female vervet monkeys show higher alcohol intake frequencies than males. Such sex differences in ethanol intake, which are just the opposite of those found in humans (see above), rhesus monkeys (11), and Syrian hamsters (12), have since been well established (13). Species is thus an important variant in determining gender-related alcohol drinking patterns.

In contrast to the positive motivational effects associated with ethanol consumption, the aversive stimulus properties of ethanol are thought to be involved in the regulation (titration) of intake. Intraperitoneal injections of ethanol can produce conditioned taste aversions; rats learn to avoid distinctively-flavored solutions paired previously with ethanol administration (14–16). Therefore, it is likely that variations in ethanol consumption may reflect underlying differences in the innate threshold of the aversive stimulus properties of ethanol.

Extensive studies have demonstrated that social, environmental, and genetic factors also contribute to the risk for alcohol dependence (3, 17). In addition, the better ability of men to metabolize and clear alcohol, resulting in lower blood alcohol concentrations usually serves as an explanation for gender differences in alcohol consumption in humans. A similar explanation may also account for sex-specific patterns of alcohol

M. Shoaib and V.K. Patchev contributed equally to this work.

Address correspondence to O.F.X. Almeida, Neuroadaptations Group, Department of Neuroendocrinology, Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, D-80804 Munich, Germany. Phone: 49-89-30622-216; FAX: 49-89-30622-461; E-mail: osa@mpipsykl.mpg.de M. Shoaib's present address is Section on Behavioural Pharmacology, Institute of Psychiatry, University of London, De Crespigny Park, London SE5 8AF, UK. V.K. Patchev's present address is Medical Research Department, Jenapharm GmbH, Otto-Schott-Str. 15, 07745 Jena, Germany.

Received for publication 14 July 1997 and accepted in revised form 13 April 1998.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/98/06/2677/09 $2.00
Volume 101, Number 12, June 1998, 2677–2685
http://www.jci.org

preference in rats: Mezey et al. (18) found gastric alcohol dehydrogenase (ADH)[1] activity to be higher in females than in males, and, consistent with the latter, Middaugh et al. (19) observed blood alcohol concentrations to be higher in males than in females after equivalent doses of ethanol (however opposite findings were reported in reference 20). An obvious question that arises from these observations is whether the enzymatic degradation of alcohol is driven by gonadal steroids. Data obtained by Vaubourdolle et al. (21) suggests an inhibition of ADH by testosterone and dihydrotestosterone in men; on the other hand, Lammers et al. (22) failed to observe a correlation between ethanol pharmacokinetics and estrogen secretion in women. Similarly, ADH activity was not altered by ovariectomy and orchidectomy in rats (18). Further, Dorgan et al., (23) did not observe a positive association between estrogen concentrations and alcohol ingestion in women. Given the apparent rise in alcohol dependence in women, and growing evidence that the medical consequences of chronic alcohol consumption may be more severe in women than in men (24–26), it seems appropriate to investigate all the possible biological mechanisms that might underlie its gender-related prevalence. While the study of sex differences in voluntary alcohol consumption in humans (4) and rats (27, 28) has been generally neglected, there is an even greater paucity of studies on brain-associated mechanisms that might account for these differences.

That sex steroids might be acting directly in the brain to influence ethanol preference was suggested by a recent study by Blanchard and Glick (29) who showed gender differences in the response of the mesolimbic dopaminergic reward system of the rat brain: female rats consumed more ethanol than males and displayed a greater release of dopamine in the nucleus accumbens, but the direct role of steroids was not addressed. In classical neuroendocrinology, sex steroids are considered to exert two types of action—organizing and activating—in the brain. Activational effects are seen throughout life, the patterns of response being determined by neural templates that are organized during early development, e.g., through sexual differentiation of the brain. In mammals, the brain is female by default unless it experiences a surge of testosterone that is subsequently aromatized to estrogen (physiological differentiation). In the rat, brain gender can be experimentally manipulated during the first 10 d of postnatal life either by castration, or pharmacologically, by the application of testosterone or estradiol (30, 31). In this study, we used the last approach to establish whether sexual differentiation of the brain, as determined by the early sex steroid milieu, might account for the typical male–female patterns of ethanol preference and ingestion in rats. In addition, we examined the activational role of sex steroids upon ethanol preference in mature rats that were gonadectomized and selectively substituted with androgen, estrogen, and/or progesterone.

Methods

Animals. Wistar rats, derived from original stock at the Max Planck Institute of Biochemistry (Martinsried, Germany), were used in these studies. Animals were housed under standard laboratory conditions (12 h light, 12 h dark cycles, with lights on at 6 A.M., 22°C, relative humidity, 60%), and maintained on ad libitum standard laboratory chow and water, unless specifically stated otherwise. Animals were group housed until 1 wk before the commencement of the oral self administration (OSA) studies, when they were individually caged. All procedures were carried out in accordance with the National Institutes of Health Guidelines on Animal Welfare, and were approved by the local animal experimentation regulatory authorities. Each treatment group consisted of six to eight animals.

OSA procedure. Animals were single caged 1 wk before testing in the OSA. At this time, all animals had reached adulthood (body weight ranges: males, 250–300 g; females, 175–200 g; neonatally-estrogenized [NE_2] females, 220–260 g). The OSA procedure used was identical to that described recently (16). Briefly, a free choice drinking procedure was used, with rats having free access to solutions of ethanol or water over a period of 23 h daily. Consumption of fluids was measured daily during a 1-h period, when bottles were weighed and filled with fresh solutions. The initial concentration of alcohol presented was increased from 2 to 4% (4 d each), followed by increases to 8 and 12% (4 d each). The positions of the two drinking bottles were changed randomly every day in order to avoid positional preferences. Ethanol preference was estimated as the percent ratio between total ethanol fluid consumption and total fluid (ethanol plus water) intake. Animals were weighed at weekly intervals, allowing computation of absolute ethanol consumption (gram/kilogram). Food was available ad libitum throughout the OSA procedure.

Experiment 1: do gonadal steroids play an organizational role in determining gender differences in alcohol preference and consumption? Groups of adult male, female, and NE_2 female rats (n = 6–8 per group) were exposed to the above-described OSA protocol. Rats used for neonatal estrogenization were delivered from timed pregnancies; litters were culled to eight pups each and pups were sexed using ano-genital distance to distinguish males from females (30). Starting on the day of birth, and on every alternate day thereafter until the postnatal day 10, female pups received 10 μg of 17β-estradiol (E_2; Sigma Chemical Co., St. Louis, MO; dissolved in sesame oil, total volume 0.1 ml) by subcutaneous injection, the site of injection being sealed with Histoacryl (Braun, Melsungen, Germany). Subgroups of female neonates and all male pups that would subsequently serve as controls were injected with oil only. Pups were weaned from their mothers at the age of 21 d and then housed according to perinatal treatment until tested in the OSA protocol. Both ethanol preference and ethanol intake relative to body weight were monitored in these animals.

Experiment 2: do the gender-specific organized patterns of ethanol drinking persist upon re-exposure to alcohol after a period of abstinence? Adult male, female, and NE_2 females (prepared as above) were exposed to the ethanol OSA paradigm. In this particular experiment, animals were presented with solutions containing ethanol at 2, 4, and 8% (4 d each); they were subsequently given a 12% ethanolic solution for 6 d, after which ethanol solutions were withdrawn for 7 d. During this period of abstinence, animals were maintained on ad libitum water. They were then re-exposed to 8% ethanol solutions (OSA procedure as before) for 6 d; the 8% ethanol concentration was chosen in view of the preceding observation of a tendency for all groups of rats to reduce their ethanol ingestion when presented with 12% ethanolic solutions. Ethanol intake was monitored during the initial and postabstinence periods.

Experiment 3: do gonadal steroids exert activational effects on ethanol consumption? This question was addressed by making comparisons of ethanol intake (OSA procedure described above) between groups of sexually mature male and female rats that were either sham operated or gonadectomized (Gdx). Surgeries were performed under

1. *Abbreviations used in this paper:* ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; AUC, area under the curve; DHT, dihydrotestosterone; E_2, 17β-estradiol; Gdx, gonadectomy; NE_2, neonatal estrogenization; OSA, oral self administration; P_4, progesterone.

Table I.

Gender	Ethanol concentration in drinking solution			
	2%	4%	8%	12%
Male	32.3±0.9	36.4±1.3	32.1±2.1	28.3±0.6
Female	43.9±2.1	55.2±1.9	36.9±1.0	30.8±0.6
NE_2 Female	41.3±2.1	44.9±2.3	37.9±1.8	38.3±1.5

Mean (±SEM) volumes of total fluid ingested (water plus ethanol) during each phase of ethanol exposure (2, 4, 8 and 12% solutions) by male, female, and NE_2 female rats during the ethanol OSA experiment (percent ethanol preference data are depicted in Fig. 1). Each gender group was comprised of eight rats; results of ANOVA on these data are given in Results (Experiment 1).

barbiturate anesthesia (Brevymital; Lilly, Bad Homburg, Germany; 30 mg/kg i.p.) 7–10 d before exposure to the OSA paradigm. Subgroups of Gdx males were given daily subcutaneous injections of 100 μg dihydrotestosterone (DHT; Sigma Chemical Co.; solubilized in sesame oil) or vehicle 1 d before and throughout the OSA procedure. All oil and steroid injections were administered between 8 and 10 A.M. Starting 1 d before, and during the entire OSA testing, subgroups of Gdx females were given subcutaneous injections of vehicle (sesame oil), 5 μg of E_2, 1 mg of progesterone (P_4; Sigma Chemical Co.) or a combination of E_2 and P_4. Ethanol intake was monitored as before.

Data presentation and statistical analysis. All data are shown as means±SEM. In some cases, cubic equations were fitted to the raw data in order to aid distinction between treatment effects in the different treatment groups (see Figs. 3–5). Standard pharmacological methods for expressing overall treatment effects, namely measures of areas under the curve (AUC) derived from the above-mentioned cubic equations, were also used in some instances (see Fig. 3). Irrespective of presentation style, all data were subjected to ANOVA and Tukey's or Dunn's post hoc multiple comparison tests, with the acceptable level of significance being preset at $P \leq 0.05$ in all tests. For analysis of effect of ethanol concentration in drinking fluid (concentration changes made in step-wise manner every 4 d), individual data from a given phase (e.g., days 1–4, 5–8, etc.) were pooled.

Results

Experiment 1. Two-way ANOVA revealed a significant effect of gender (male, female, and NE_2 female) on ethanol preference ratios ($F = 141.69$, $df = 2, 84$; $P < 0.001$), as well as of ethanol concentration in drinking solutions (2, 4, 8, or 12%

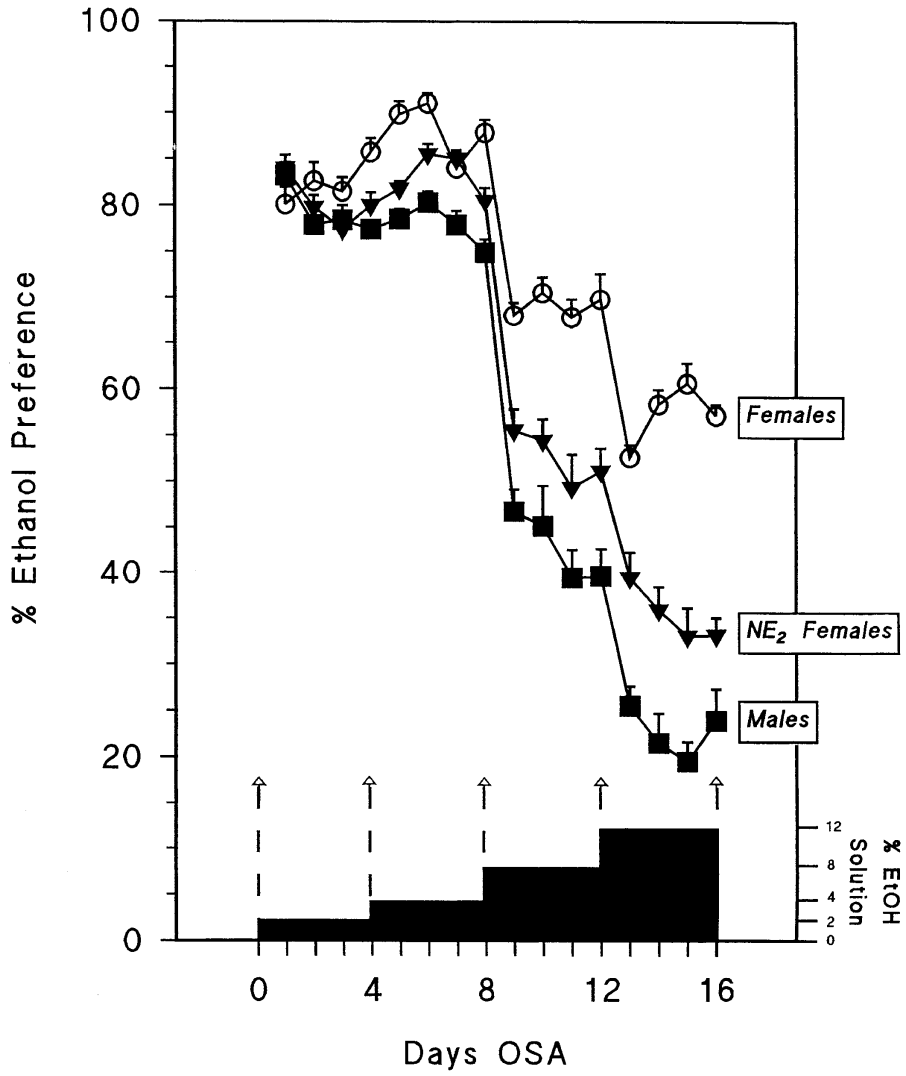

Figure 1. Ethanol preference profiles for male, female, and NE_2 female rats (means±SEM are shown; $n = 8$ rats per treatment group). Animals were exposed to an ethanol OSA paradigm in which alcohol concentrations were increased step-wise every 4 d (*black squares*). Tukey's multiple comparison test revealed the following significant differences ($P < 0.05$): 4% ethanol solution: males versus females; 8 and 12% ethanol solutions: all gender groups significantly different from each other. All other statistical differences are described in the text.

ethanol; $F = 523.64$, $df = 3, 84$; $P < 0.001$). Further, a significant interaction between gender and treatment was detected ($F = 26.05$, $df = 6, 84$; $P < 0.001$). The total volumes of fluid (water plus ethanol) consumed by each gender group during each 4-d phase of exposure to the different ethanol concentrations are shown in Table I. Analysis of those data (Kruskall-Wallis ANOVA) detected significant gender-related differences in fluid consumption ($H = 67.2$, $df = 2$; $P < 0.001$). Significant differences in the amounts of fluid ingested during exposure to the different concentrations of ethanol were also found within each gender group (males: $H = 26.04$, $df = 3$, $P < 0.001$; females: $H = 74.24$, $df = 3$, $P < 0.001$; NE_2 females: $H = 7.93$, $df = 3$, $P < 0.05$).

Adult male, female, and NE_2 female rats displayed similar preferences (around 80%; $P \geq 0.05$) for alcohol when presented at concentrations of 2 and 4% (Fig. 1). When the concentration of ethanol was increased to 8% and above, all groups of rats showed a marked decline in their preference for ethanolic solutions ($P < 0.05$ for all gender groups). At these higher concentrations, however, major differences in preference rates between the different gender groups became discernible, with females showing significantly higher preference scores as compared with males ($P < 0.05$), but also to NE_2 females ($P < 0.05$). Ethanol preference was significantly higher in the NE_2 female group as compared with males ($P \leq 0.05$).

An analysis of absolute daily ethanol consumption revealed that females consumed significantly greater amounts of ethanol (relative to body weight) than males and NE_2 females throughout the OSA procedure ($P \leq 0.05$; Fig. 2). During the initial phases of ethanol presentation, NE_2 females consumed significantly more ethanol than males ($P \leq 0.05$), although the difference between the two groups was abolished with increasing duration of exposure to the OSA protocol, i.e., at higher concentrations of ethanol in the drinking fluid. Interestingly,

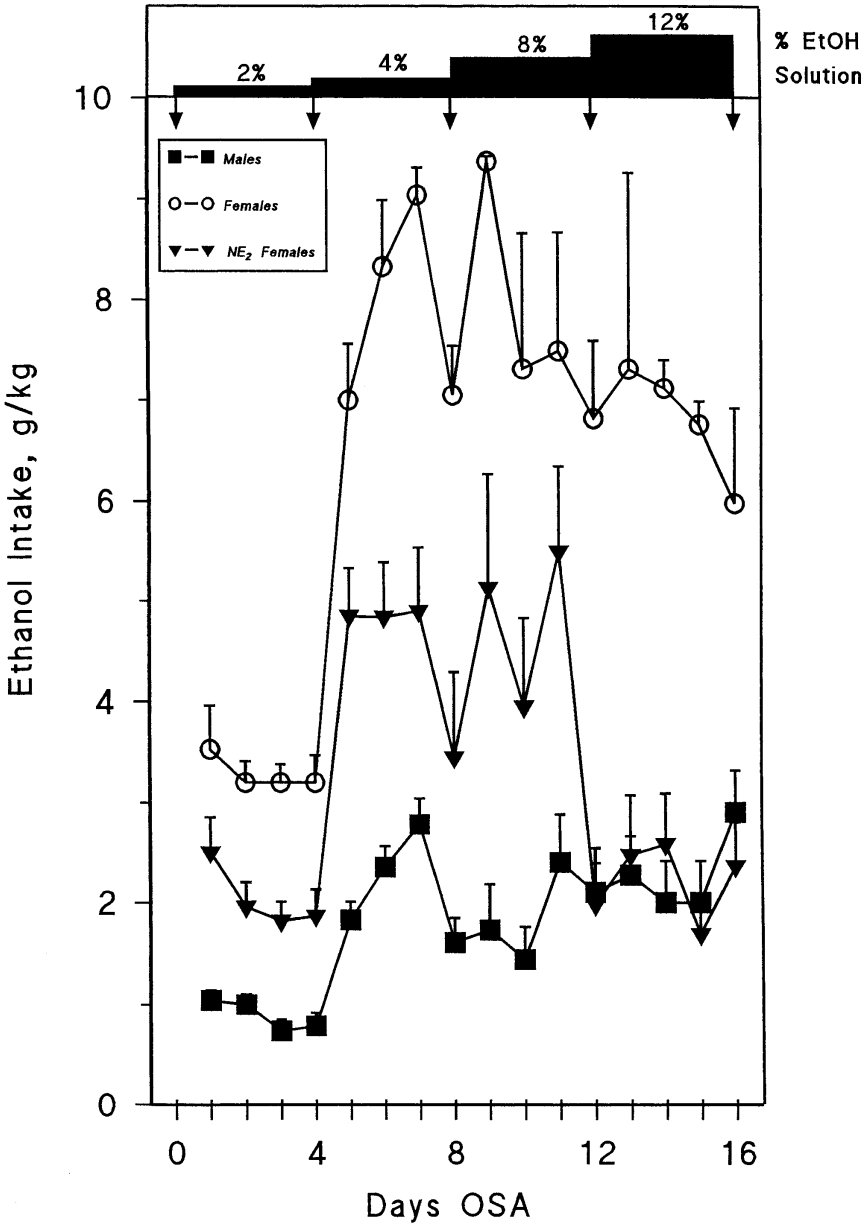

Figure 2. Daily ethanol ingestion by male, female, and NE_2 female rats during ethanol self-administration. The data shown here are from the same set of animals for which ethanol preference profiles are shown in Fig. 1. Data refer to means±SEM, with $n = 8$ rats per treatment group. All statistical differences are described in the text.

all three groups showed an ability to regulate their daily ethanol intake to a relatively constant level (~ 7g/kg in females, and 2-4 g/kg for males and NE_2 females), despite the gradual increases in the concentration of ethanol in the drinking solutions.

Experiment 2. During first exposure to the OSA paradigm, groups of males, females, and NE_2 females showed ethanol intake profiles that were similar to those obtained in Experiment 1 (compare Figs. 2 and 3). A two-way ANOVA disclosed significant effects of gender on ethanol intake per kilogram body weight ($F = 61.5$, $df = 2, 80$; $P < 0.001$), as well as of ethanol concentration (exposure to 2, 4, 8 or 12% ethanol; $F = 22.35$, $df = 3, 80$; $P < 0.001$). In addition, significant gender × ethanol concentration interactions were detected ($F = 4.99$, $df = 6, 80$; $P < 0.001$). Pairwise multiple comparison tests revealed the following significant differences ($P < 0.05$): male versus female, male versus NE_2 females, and females versus NE_2 females. Based on AUC computations, females were found to consume approximately three times as much ethanol as males, and approximately 1.6 times more than NE_2 females. After a 1-wk period of abstinence, animals were reintroduced to the OSA procedure with alcohol being presented at a concentration of 8%. All groups of animals reinstated ethanol drinking at levels (in terms of grams ethanol ingested per kilogram) that were statistically comparable ($P > 0.05$) to those seen during the previous exposure to 8% ethanolic solutions. Gender proved to exert a significant influence over postabstinence ethanol ingestion ($H = 18.98$, $df = 2$; $P < 0.001$). Pairwise comparisons revealed significant differences in postabstinence ethanol intake between males versus females and females versus NE_2 females ($P < 0.05$); there were no significant differences between amounts ingested by males and NE_2 females. Further, the relative differences between the groups, as judged by AUC measurements, showed a rank order similar to that seen during the initial OSA procedure (male and NE_2 female ethanol intake being, respectively, 2.4 and 2 times lower than that of females).

Experiment 3. To address the question of whether gonadal steroids secreted during adulthood might influence ethanol drinking, intact male and female rats, as well as Gdx males and females that received either vehicle (oil) or sex steroid hormone replacement therapy: males, DHT; females, either E_2, P_4, or E_2 and P_4) were exposed to the OSA procedure.

In males, the gonadal steroid milieu proved to exert significant effects upon ethanol consumption ($F = 24.61$, $df = 2, 36$;

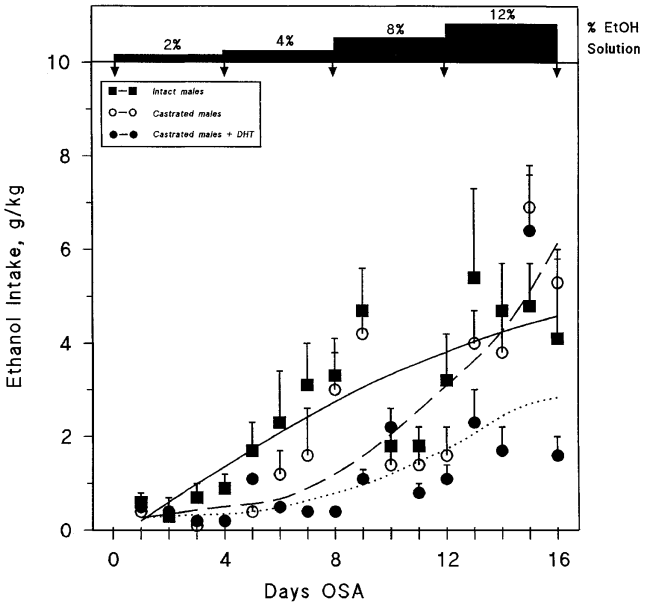

Figure 4. Comparison of ethanol ingestion by intact, castrated, and DHT-supplemented castrated male rats during exposure to an OSA paradigm in which animals were presented with a free choice of drinking solutions containing water or increasing concentrations of ethanol. Actual data points shown are means±SEM (n = 6–8 animals per group). The line plots are based on cubic fits to the raw data. All statistical differences are described in the text.

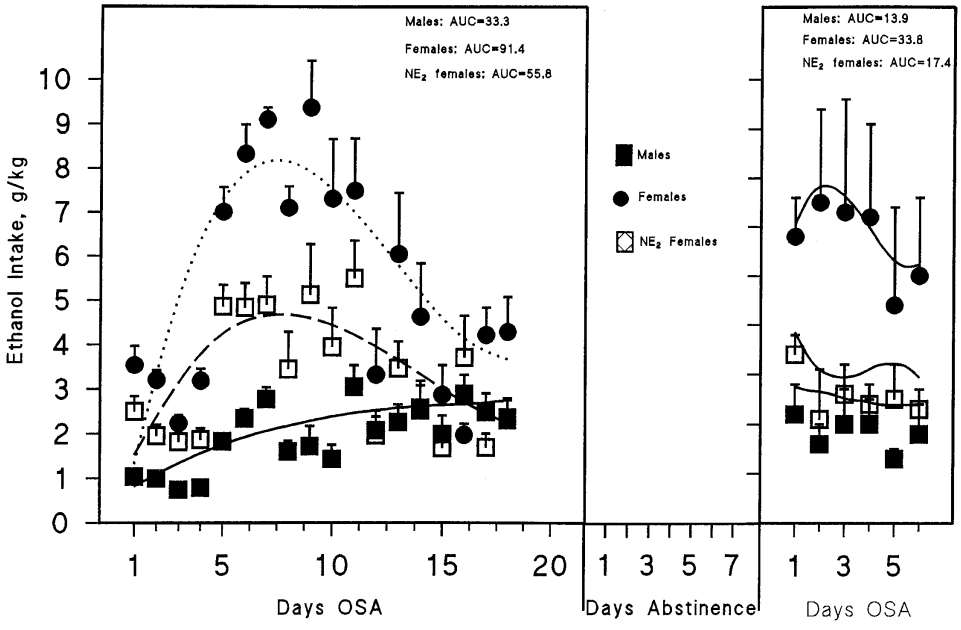

Figure 3. Amounts of ethanol ingested by male, female, and NE_2 female rats during a first exposure to an ethanol OSA paradigm, and after a second exposure to the OSA after a 7-d period of abstinence. The OSA protocol used in this experiment consisted of presentations with solutions containing 2% (days 1–4), 4% (days 5–8), 8% (days 9–12), and 12% (days 13–18) of ethanol; after the abstinence period, rats were presented with solutions containing 8% ethanol. During all phases of the experiment, bottles containing water only were also freely available to the animals. Actual data points shown are means±SEM (n = 6–8 animals per group); the line plots are based on cubic fits to the raw data from which areas under the curve (AUC) were further derived. All statistical differences are described in the text.

$P = 0.03$; Fig. 4). Pairwise comparison testing revealed that although intact and castrated rats did not differ in their ethanol intake, castrated DHT-treated rats drank significantly smaller amounts of ethanol than intact and castrated controls ($P < 0.05$).

Overall, the various hormone manipulations in female rats produced significant effects on ethanol ingestion ($F = 7.589$, $df = 4, 44$; $P < 0.001$). Nevertheless, application of Tukey's test for multiple pairwise comparisons revealed a complex pattern of influence of the sex steroid milieu upon ethanol intake. As shown in Fig. 5 *a*, ovariectomized females displayed large fluctuations in their daily ethanol intake, and, although they tended to show an overall reduction in drinking solutions containing 12% ethanol, post hoc comparisons between the data for intact and ovariectomized rats failed to reach significance ($P > 0.05$). As compared with intact females, ovariectomized rats that were supplemented with E_2 showed a significant decrease in their ethanol consumption ($P < 0.05$; Fig. 5 *b*); however, their ethanol ingestion was not significantly greater than that observed in untreated ovariectomized females ($P > 0.05$). Ovariectomized rats receiving P_4 replacement therapy did not drink significantly different amounts of ethanol as compared with their gonad-intact counterparts (Fig. 5 *c*; $P > 0.05$); they also did not differ significantly in this respect from untreated and E_2-treated ovariectomized rats ($P > 0.05$). Ethanol ingestion was significantly lower in ovariectomized rats given combined E_2 and P_4 treatment than in intact female rats (Fig. 5 *d*; $P < 0.05$) and in untreated ovariectomized rats ($P < 0.05$). However, ethanol ingestion was not significantly different between the E_2/P_4–treated, E_2-treated, and P_4-treated groups ($P > 0.05$).

Discussion

Using an OSA procedure, we confirmed previous reports (13, 32, 33) that male and female rats differ markedly in their ingestion of ethanol, with females consuming significantly greater amounts of the substance (however, see reference 34). Fur-

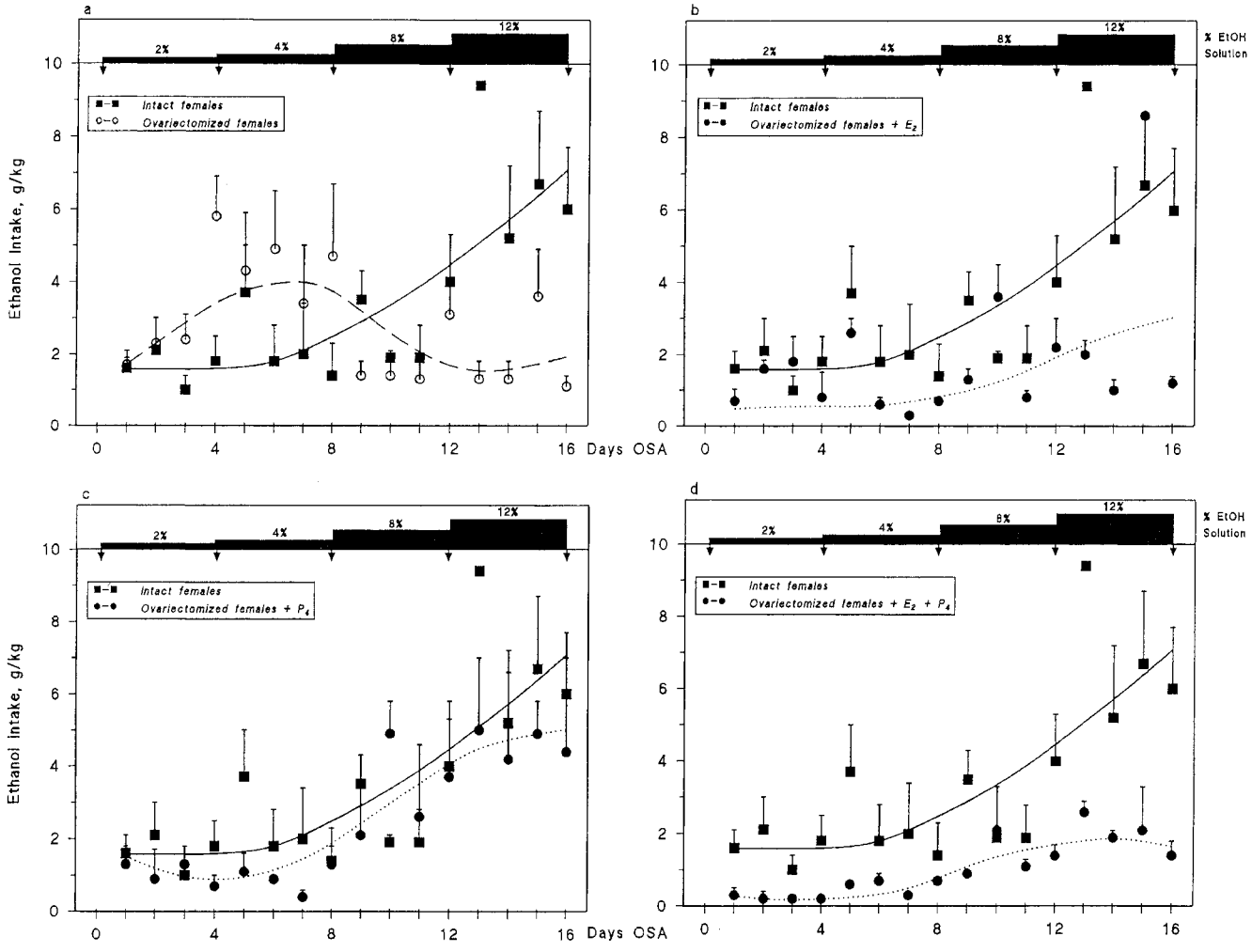

Figure 5. Comparison of ethanol ingestion by intact female (*a–d*), ovariectomized (*a*), E_2-supplemented ovariectomized (*b*), P_4-supplemented ovariectomized (*c*), and E_2+P_4–supplemented ovariectomized (*d*) rats. All treatment groups were exposed to an OSA paradigm in which animals were presented with a free choice of drinking solutions containing water or increasing concentrations of ethanol. Actual data points shown are means±SEM (n = 6–8 animals per group). The line plots are based on cubic fits to the raw data. All statistical differences are described in the text.

ther, we established that the larger amounts of ethanol ingested by females had a behavioral correlate insofar that female rats showed markedly higher ethanol preference scores in the presence of another drinking fluid of choice (water) and without food deprivation. The latter may be taken to indirectly indicate the positive reinforcing properties of the alcohol delivered by OSA (compare references 35–38). Interestingly, males and females showed the ability to regulate or titrate their alcohol intake with increasing ethanol concentrations in the drinking solution, although females appeared to be worse in this respect (33), possibly reflecting gender differences in sensitivity to the aversive stimulus properties of ethanol. The gender differences observed here may be accounted for, at least partially, by the fact that female rats have been demonstrated to be able to metabolize and clear alcohol more effectively than males (18). Thus, on one hand, their exposure to the adverse central and peripheral effects of alcohol is reduced, on the other hand, they need to drink more in order to maintain a given blood alcohol level. However, it is worth noting that, although alcohol exerts very potent effects on CNS function, and often in a gender-specific mode (25, 29, 39, 40–42), sex differences do not exist in the activities of ADH and acetaldehyde dehydrogenase (ALDH) in the brain (43).

The addictive potential of alcohol, like that of other drugs of abuse, is believed to be based on its motivational properties (38). There is now evidence that the latter are mediated by the mesolimbic dopamine system (44). In a recent study, Blanchard and Glick (29) showed that ethanol-stimulated dopamine release in the nucleus accumbens is more pronounced in female rats than in male rats. This finding strongly suggests that alcohol-sensitive CNS substrates may be sexually differentiated in a fashion analogous to that seen for several neuroendocrine secretions and behaviors (31), and served as the rationale for a major part of these studies. Our experiments using NE_2 as a means to phenotypically masculinize the female brain (30, 31) strongly suggest that sex differences in alcohol preference and consumption result from the organization of the brain during early development. Specifically, we observed that NE_2 female rats, like their male counterparts, show ethanol drinking profiles that are distinct from those displayed by normal female rats (lower preference, lower absolute intake). In all cases, the data for the NE_2 females were found to be intermediate between those obtained for males and females, a typical finding in experiments of this nature (31, 45) that most probably reflects the inability to completely masculinize the undifferentiated brain (30). We also showed that, irrespective of whether sexual differentiation occurs physiologically or is induced experimentally, the gender-specific patterns of ethanol intake seen during initial exposure to the substance continue to be expressed even after a period of abstinence. These results provide a novel view on the mechanism by which sex-related patterns in ethanol drinking patterns can be accounted for.

Since certain hepatic enzymatic systems (but not necessarily ADH and ALDH) are differentiated under the control of developmentally secreted sex steroids (46), it is necessary to consider the possibility that our NE_2 manipulation might have resulted in a masculinization of the ADH and ALDH systems, i.e., our suggestion that sexual differentiation of the brain is the principal factor may be incorrect. In interpreting our findings, it is also necessary to consider the possibility that, rather than altering ethanol preference per se, our NE_2 procedure might have altered this parameter by differentiating the neural circuits responsible for sex differences in taste reactivity. As reviewed by van Haaren (47), gender differences in taste reactivity may represent an important factor in determining alcohol preference although the mechanisms must still be explored more thoroughly.

Besides their important organizational effects during development, sex steroids also act in a so-called activational capacity throughout life (31, 45). The latter effects occur not only in the brain but also at peripheral targets such as the liver. Further, ethanol exerts a major influence upon gonadal steroid secretion in both humans and animals (48, 49). An experiment was thus conducted to examine whether ethanol intake is influenced by manipulations of the sex steroid milieu during adulthood, i.e., when the brain is fully sexually differentiated. Neither ovariectomy nor castration affected ethanol ingestion. When considered together with the results of studies by Mezey et al. (18) showing that ADH activity is unaffected by gonadectomy, our observations tend to argue against a metabolic basis for these different responses. On the other hand, the finding that DHT treatment of castrated rats resulted in an inhibition of ethanol ingestion can possibly be accounted for by this steroid's potent inhibitory effects upon alcohol metabolizing enzymes in vitro (21, 50). If applicable in vivo, DHT-treated animals would experience high blood alcohol concentrations over an extended period. In contrast to testicular androgens such as DHT, estrogens have not been found to alter ADH activity in rats (50). A comprehensive review of the literature on alcohol pharmacokinetics in women in different phases of the menstrual cycle also failed to find any dependence of this parameter on sex hormone levels (22, 51). Unfortunately, the results of our investigation on the effects of E_2 and/or P_4 replacement therapy in ovariectomized rats proved difficult to interpret. Surprisingly, ovariectomized rats treated with E_2 alone showed a decrease in ethanol ingestion as compared with intact (but not ovariectomized) rats, and ovariectomized rats treated with a combination of E_2 and P_4 consumed less ethanol than intact and nonsteroid-treated ovariectomized rats. Since P_4 did not exert any significant effect on its own, it seems warranted to conclude that E_2 does have a modulatory effect on ethanol preference, the manifestation of which is subtly determined by the presence or absence of P_4 and/or some other ovarian factor. These apparently complex interactions between E_2 and P_4 may underlie previous unsuccessful attempts to draw convincing correlations between menstrual fluctuations in E_2 and P_4 secretion with alcohol consumption in women (23). Given reports that women may consume larger amounts of alcohol during the premenstruum, when blood P_4 titers are rising (52), it seems imperative that more studies are conducted on examining the role of ovarian steroids on alcohol seeking behavior.

In summary, we have confirmed previous findings that rats display pronounced gender-related differences in the consumption of alcohol, with females ingesting approximately twofold greater amounts of ethanol in an OSA procedure. These differences, which agree with a behavioral measure of alcohol preference, can be modulated by the neonatal gonadal steroid environment, a finding highly supportive of the view that they may emerge from sexually differentiated or organized brain mechanisms. The results presented here point to the impact that sex steroids can have during early brain development and differentiation on subsequent alcohol-seeking be-

havior. Apparently complex interactions were also demonstrated between gonadal steroids and ethanol ingestion, and it was postulated that the majority of these most likely occur at a central site rather than in the metabolic pathways leading to alcohol elimination. Although the relationship between gender and ethanol consumption in humans is inverse to that in rats, the results presented here may have consequences for furthering our knowledge—beyond the common explanations based on alcohol metabolism—on why men ingest more ethanol than women. For example, and especially in view of the changing ratio of alcoholism in men and women (see Introduction), the possibility that prenatal exposure to estrogens, which have the potential to alter sexual differentiation of the brain, may play a role in the changing demographic patterns of alcoholism needs to be considered. This aspect may be particularly relevant in the context of unknowing exposure to the so-called environmental estrogens.

Acknowledgments

This work was supported by a Health Research 2000 grant (FKZ: 01EB9419/8) from the German Ministry of Education & Science, and a European Training Fellowship (M. Shoaib) from the Wellcome Trust.

References

1. de Lint, J., and W. Schmidt. 1971. The epidemiology of alcoholism. *In* Biological Basis of Alcoholism. Y. Israel and J. Mardones, editors. John Wiley & Sons, Inc., New York. 423–442.

2. Grant, B.F., T.C. Harford, P. Chou, R. Pickering, D. Dawson, F. Stinson, and J. Noble. 1991. Prevalence of DSM-III-R alcohol abuse and dependence: United States, 1988. *Alcohol Health Res. World.* 15:15–20.

3. Wilsnack, S.C., and R.W. Wilsnack. 1995. Drinking and problem drinking in US women. *In* Recent Developments in Alcoholism, Vol. 12. M. Galanter, editor. Plenum Publishing Corp., New York. 29–60.

4. Gomberg, E.S. 1993. Recent developments in alcoholism: gender issues. *Recent Dev. Alcohol.* 11:95–107.

5. Kidorf, M., A.R. Lang, and W.E. Pelham. 1990. Beverage preference, beverage type and subject gender as determinants of alcohol consumption in the laboratory. *J. Stud. Alcohol.* 51:331–335.

6. Dawson, D.A., and L.D. Archer. 1993. Relative frequency of heavy drinking and the risk of alcohol dependence. *Addiction.* 88:1509–1518.

7. Segovia-Riquelme, N., A. Varela, and J. Mardones. 1971. Appetite for alcohol. *In* Biological Basis of Alcoholism. Y. Israel and J. Mardones, editors. John Wiley & Sons, Inc., New York. 299–334.

8. Eriksson, K. 1968. Genetic selection for voluntary alcohol consumption in albino rats. *Science.* 159:739–740.

9. Eriksson, K., and P.H. Pikkarainen. 1968. Differences between the sexes in voluntary alcohol consumption and liver ADH-activity in inbred strains of mice. *Metabolism.* 17:1037–1042.

10. Juarez, J., C. Guzman-Flores, F.R. Ervin, and R.M. Palmour. 1993. Voluntary alcohol consumption in vervet monkeys: individual, sex and age differences. *Pharmacol. Biochem. Behav.* 46:985–988.

11. Grant, K.A., and C.E. Johanson. 1988. Oral ethanol self-administration in free-feeding rhesus monkeys. *Alcohol Clin. Exp. Res*. 12:780–784.

12. Moss, H.B., R.J. Salin-Pascual, P. Rathnagiri, D. Goldman, and L. Tamarkin. 1987. Sex differences in ethanol sensitivity and alcohol and aldehyde dehydrogenase activities in the Syrian hamster. *Alcohol Drug Res.* 7:301–307.

13. Lancaster, F.E., T.D. Brown, K.L. Coker, J.A. Elliott, and S.B. Wren. 1996. Sex differences in alcohol preference and drinking patterns emerge during the early postpubertal period. *Alcohol Clin. Exp. Res*. 20:1043–1049.

14. Froehlich, J.C., J. Harts, L. Lumeng, and T.-K. Li. 1988. Differences in response to the aversive properties of ethanol in rats selectively bred for oral ethanol preference. *Pharmacol. Biochem. Behav.* 31:215–222.

15. Gauvin, D.V., and F.A. Holloway. 1992. Ethanol tolerance developed during intoxicated operant performance in rats prevents subsequent ethanol-induced conditioned taste aversion. *Alcohol.* 9:167–170.

16. Shoaib, M., and O.F.X. Almeida. 1996. Absence of tolerance to the aversive stimulus properties of ethanol following oral ethanol self-administration. *Alcohol.* 13:175–180.

17. National Institute of Drug Abuse/National Institute on Alcohol Abuse and Alcoholism. 1989. Highlights from the 1987 National Drug and Alcoholism Treatment Unit Survey (NDATUS). NIDA/NIAAA, Rockville, MD. 100 pp.

18. Mezey, E., S. Sharma, L. Rennie, and J.J. Potter. 1992. Sex differences in gastric alcohol dehydrogenase activity of Sprague-Dawley rats. *Gastroenterology.* 103:1804–1810.

19. Middaugh, L.D., W.F. Frackelton, W.O. Boggan, A. Onofrio, and C.L. Shepherd. 1992. Gender differences in the effects of ethanol on C57BL/6 mice. *Alcohol.* 9:257–260.

20. Rivier, C. 1993. Female rats release more corticosterone than males in response to alcohol: influence of circulating sex steroids and possible consequences for blood alcohol levels. *Alcohol Clin. Exp. Res.* 17:854–859.

21. Vaubourdolle, M., J. Guechot, O. Chazouilleres, R.E. Poupon, and J. Giboudeau. 1991. Effect of dihydrotestosterone on the rate of ethanol elimination in healthy men. *Alcohol Clin. Exp. Res*. 15:238–240.

22. Lammers, S.M., D.E. Mainzer, and M.H. Breteler. 1995. Do alcohol pharmacokinetics in women vary due to the menstrual cycle? *Addiction.* 90:23–30.

23. Dorgan, J.F., M.E. Reichman, J.T. Judd, C. Brown, C. Longcope, A. Schatzkin, W.S. Campbell, C. Franz, L. Kahle, and P.R. Taylor. 1994. The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control.* 5: 53–60.

24. Glenn, S.W. 1993. Sex differences in alcohol-induced brain damage. *In* Alcohol-induced Brain Damage. *NIAAA Research Monograph.* 22:195–212.

25. Harper, C.G., N.A. Smith, and J.J. Krill. 1990. The effects of alcohol on the female brain: a neuropathological study. *Alcohol.* 25:445–448.

26. Norton, R., T. Dwyer, and S. MacMahon. 1987. Alcohol consumption and the risk of alcohol related cirrhosis in women. *Br. Med. J*. 295:80–82.

27. Lancaster, F.E. 1994. Gender differences in the brain: implications for the study of human alcoholism. *Alcohol Clin. Exp. Res.* 18:740–746.

28. Li, T.-K., and L. Lumeng. 1984. Alcohol preference and voluntary alcohol intakes of inbred rat strains and the National Institutes of Health heterogenous stock of rats. *Alcohol Clin. Exp. Res*. 8:485–486.

29. Blanchard, B.A., and S.D. Glick. 1995. Sex differences in mesolimbic dopamine responses to ethanol and relationship to ethanol intake in rats. *Rec. Dev. Alcohol* 12:231–241.

30. Goy, R.W., and B.S. McEwen. 1980. Sexual differentiation of the brain. MIT Press, Cambridge, MA. 223.

31. Patchev, V.K., S. Hayashi, C. Orikasa, and O.F.X. Almeida. 1995. Implications of estrogen-dependent brain organization for gender differences in hypothalamo-pituitary-adrenal regulation. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 9: 419–423.

32. Adams, N. 1995. Sex differences and the effects of tail pinch on ethanol drinking in Maudsley rats. *Alcohol.* 12:463–468.

33. Lancaster, F.E., and K.S. Spiegel. 1992 Sex differences in pattern of drinking. *Alcohol.* 9:415–420.

34. van Haaren, F., and K. Anderson. 1994. Sex differences in schedule-induced alcohol consumption. *Alcohol.* 11:35–40.

35. Cicero, T.J. 1979. A critique of animal models of alcohol self-administration, preference, tolerance and physical dependence. *In* The Pharmacology of Ethanol. E. Majchrowicz, editor. Plenum Publishing Corp., New York. 553–560.

36. Keane, B., and B.E. Leonard. 1989. Rodent models of alcoholism: a review. *Alcohol.* 24:299–309.

37. Neill, J.C., A.M. Domeney, and B. Costall. 1994. Initiation and maintenance of ethanol oral self-administration in female Sprague-Dawley rats. *Alcohol.* 11:207–218.

38. Altman, J., B.J. Everitt, S. Glautier, A. Markou, D. Nutt, R. Oretti, G.D. Phillips, and T.W. Robbins. 1996. The biological, social and clinical bases of drug addiction: commentary and debate. *Psychopharmacology.* 125:285–345.

39. Lisciotto, C.A., J.F. DeBold, and K.A. Miczek. 1990. Sexual differentiation and the effects of alcohol on aggressive behavior in mice. *Pharmacol. Biochem. Behav.* 35:357–362.

40. Haut, J.S., B.E. Beckwith, T.V. Petros, and S. Russell. 1989. Gender differences in retrieval from long-term memory following acute intoxication with ethanol. *Physiol. Behav.* 45:1161–1165.

41. Niaura, R.S., P.E. Nathan, W. Frankenstein, A.P. Shapiro, and J. Brick. 1987. Gender differences in acute psychomotor, cognitive, and pharmacokinetic response to alcohol. *Addict. Behav*. 12:345–356.

42. Linnoila, M., C.W. Erwin, W.P. Cleveland, P.E. Logue, and W.D. Gentry. 1978. Effects of alcohol on psychomotor performance of men and women. *J. Stud. Alcohol.* 39:745–758.

43. Messiha, F.S. 1985. Neurotoxicological analyses of voluntary alcohol drinking by the rat. *Neurobehav. Toxicol. Teratol.* 7:155–159.

44. Tabakoff, B., and P.L. Hoffman. 1996. Alcohol addiction: an enigma among us. *Neuron.* 16:909–912.

45. Almeida, O.F.X., V. Canoine, S. Ali, F. Holsboer, and V.K. Patchev. 1997. Activational effects of gonadal steroids on hypothalmo-pituitary-adrenal regulation in the rat disclosed by response to dexamethasone suppression. *J. Neuroendocrinol.* 9:129–134.

46. Zaphiropoulos, P.G., A. Mode, G. Norstedt, and J.-Å. Gustafsson. 1989. Regulation of sexual differentiation in drug and steroid metabolism. *Trends Pharmacol. Sci.* 10:149–153.

47. van Haaren, F., 1995. Experimental analysis of sex differences in alcohol consumption. *In* Stress, Gender, and Alcohol-Seeking Behavior. *NIAAA Re-*

search Monograph. 29:293–310.

48. Emanuele, M.A., M.M. Halloran, S. Uddin, J.J. Tentler, N.V. Emanuele, A.M. Lawrence, and M.R. Kelley. 1993. The effects of alcohol on the neuroendocrine control of reproduction. *In* Alcohol and the Endocrine System. *NIAAA Research Monograph.* 23:89–166.

49. Mello, N.K., J.J. Mendelson, and S.K. Teoh. 1993. An overview of the effects of alcohol on neuroendocrine function in women. Alcohol and the endocrine system. *NIAAA Research Monograph.* 23:139–169.

50. Teschke, R., and B. Wiese. 1982. Sex dependency of hepatic alcohol metabolizing enzymes. *J. Endocrinol. Invest.* 5:243–250.

51. Cole-Harding, S., and J. Wilson. 1987. Ethanol metabolism in men and women. *J. Stud. Alcohol.* 48:380–387.

52. Mello, N.K., J.H. Mendelson, and B.W. Lex. 1990. Alcohol use and premenstrual symptoms in social drinkers. *Psychopharmacology.* 101:448–455.