ELSEVIER

Pharmacology, Biochemistry and Behavior 67 (2000) 801–808

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

# Genetic, sex, and early environmental effects on the voluntary alcohol intake in Wistar rats

F. Sluyter[a,*], M. Hof[b], B.A. Ellenbroek[b], S.B. Degen[b], A.R. Cools[b,1]

[a]*Department of Biological Psychology, Vrije Universiteit, Vander Boechorststraa 1, 1081 BT Amsterdam, Netherlands*
[b]*Department of Psychoneuropharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, Netherlands*

Accepted 8 April 2000

**Abstract**

The aim of this study was to investigate the effects of genetic, sex, and early environmental factors on the voluntary alcohol intake in Wistar rats. Genetic correlates were examined by comparing animals pharmacogenetically selected for high susceptibility to apomorphine (APO-SUS) with animals selected for low susceptibility (APO-UNSUS). Early environmental factors were investigated through postnatal manipulations (cross-fostering in APO-SUS and maternal deprivation in APO-UNSUS). Voluntary alcohol intake was measured using a two-bottle, free-choice protocol, in which animals could choose either water or an ascending series of alcohol concentrations every second day. Genetic correlates were only observed in male rats, with APO-UNSUS animals consuming more alcohol than APO-SUS animals. No effect of the early postnatal manipulations was detected: neither cross-fostering nor maternal deprivation influenced the voluntary alcohol intake. As for the influence of gender on ethanol self-administration, APO-SUS females consume more alcohol than APO-SUS males, while no sex differences were observed in APO-UNSUS animals. 

*Keywords:* Alcohol; Selected lines; Dopamine; Apomorphine; Genetics; Maternal environment; Cross-fostering; Maternal deprivation; Gender; Sex differences; Wistar; Rats

## 1. Introduction

It has long been accepted that genetic factors are important in the etiology of alcoholism. As early as 1960, Kaij [16] reported that identical twin pairs show significantly greater similarities when it comes to social problems with alcohol than fraternal twins. Ever since, many studies have been performed using various methods and the evidence is fairly consistent that genetic variation contributes to individual differences in alcohol abuse or dependence. In addition to human twin studies, animal models have been developed. McClearn and Rodgers [22] were one of the first to show that genes affect the intake of alcohol in mice. Since then, selective breeding experiments have demonstrated that the voluntary intake, the sensitivity, and the withdrawal from alcohol are under genetic control (see Ref. [24] for an overview of alcohol-related selected lines).

The development of molecular techniques, particularly targeted gene disruption (knockout), have shed more light on the neurobiological substrates that are involved in the distinct stages that lead to alcohol abuse or dependence. Since dopaminergic transmission plays a crucial role in the mediation of rewarding properties of drugs of abuse, including alcohol (see, among others, Ref. [17]), dopamine receptor subtypes have been a focus of the targeted gene disruption method. Results from knockout studies suggest the involvement of both the dopamine D1 and D2 receptors in alcohol-seeking behavior. Mice lacking either the D1 or the D2 receptor show reduced levels of voluntary alcohol intake [7,23].

The first aim of this paper is to expand on the association between dopamine receptors and voluntary alcohol intake (or, more technically, ethanol self-administration), and to investigate whether rat lines that differ in dopaminergic neurotransmission also differ in alcohol consumption. The lines in this study were bidirectionally selected for susceptibility to apomorphine, a dopamine

* Corresponding author. Tel.: +31-20-4448787; fax: +31-20-4448832.
*E-mail address*: f.sluyter@psy.vu.nl.
[1] For reprint requests. Tel.: +31-24-3613693; fax: +31-24-3540044.
*E-mail address*: a.cools@prf.kunnl.

0091-3057/00/$ – see front matter 
PII: S0091-3057(00)00425-1

agonist that acts on both the D1 and D2 receptors, but primarily on the latter. Selective breeding has resulted in a rat line susceptible to apomorphine (APO-SUS, with high stereotyped gnawing scores) and a line unsusceptible to apomorphine (APO-UNSUS, with low stereotyped gnawing scores) [5]. It is important to emphasize that APO-SUS and APO-UNSUS were selected from an outbred Wistar population and are therefore, as opposed to knockouts, not artificially created mutants. The APO-SUS and APO-UNSUS selected lines represent the extremes of naturally occurring variation in dopaminergic activity. Moreover, susceptibility to apomorphine is not the only difference between these lines. They vary for many neurobehavioral, pharmacological, and endocrinological variables (see Ref. [6]), in such a consistent manner that one can only conclude that each line is marked by its own specific structure, function, and reactivity of brain and body [5,6].

One of the fundamental behaviors in which the APO-SUS and APO-UNSUS lines differ is their reaction to novelty. APO-SUS animals show a robust response when first exposed to an open field, continuing their exploratory behavior for some length of time, which results in long distances traveled and long habituation times. APO-UNSUS, on the other hand, start exploring the novel environment but stop exploring much sooner, which results in short distances traveled and short habituation times. Previous experiments in our laboratory have demonstrated that male Wistar rats initially classified as high responders to novelty (HR) drink less alcohol than those classified as low responders to novelty (LR) [14]. Accordingly, given the association between response to novelty and apomorphine, along with the association between response to novelty and alcohol preference, we hypothesize that APO-SUS males will consume less alcohol than APO-UNSUS males.

Environmental factors are also important in the development of voluntary alcohol intake. For instance, social housing conditions, such as crowding and isolation increase alcohol consumption in animals. Relatively little research has addressed early life events, especially the effects of the maternal environment on alcohol preference later in life. The maternal environment has elicited attention with respect to fetal alcohol syndrome, in which behavioral and cognitive deficits result from continuous alcohol exposure during gestation and lactation. However, the maternal environment has not often attracted attention as a source of variation itself. Hence, the second aim of this study was to investigate the effect of the maternal environment on voluntary alcohol intake. Toward this end, we manipulated the maternal environment of newborn rats and measured alcohol self-administration of the same rats at adult age. We performed two distinct experiments: one in APO-SUS, another one in APO-UNSUS. In the first experiment, we fostered APO-SUS litters to APO-UNSUS mothers (cross-fostering). In addition to a normal control group, which was left undisturbed at birth, an extra group was added in which APO-SUS litters were in-fostered (litters were moved to another mother who was also from the APO-SUS selected line). In the second experiment, APO-UNSUS litters were taken from their mother for 24 h at day 9 of age (maternal deprivation). This two-sided procedure (cross-fostering in one line, maternal deprivation in the other) has been shown to influence the original selection criterion, i.e., the susceptibility to apomorphine. That is, APO-SUS rats are sensitive to cross-fostering but not to maternal deprivation; conversely, APO-UNSUS animals are sensitive to maternal deprivation, but not to cross-fostering. We hypothesize, therefore, that cross-fostering increases alcohol preference in APO-SUS rats, whereas maternal deprivation decreases alcohol preference in APO-UNSUS rats [9].

The third aim of this study is to examine whether female rats drink the same amount of alcohol as males. More importantly, do the expected differences between APO-SUS and APO-UNSUS males also apply for APO-SUS and APO-UNSUS females, or is the genetic variation gender-specific? Furthermore, do the early postnatal manipulations produce similar results in males and in females? To answer these questions, females were included in all experiments.

Voluntary alcohol intake was measured as described previously [14]. Briefly, starting with a 2% alcohol solution in a two-bottle, free-choice paradigm, every second day the alcohol concentration was increased by 1%, up to 10%, inclusive. In between drinking days, animals were exposed to water only. Similar to alcohol deprivation, alternate day access increases voluntary alcohol intake in rats.

## 2. Materials and methods

### 2.1. Housing

All animals were born and bred in the Central Animal Laboratory of the Catholic University of Nijmegen. After weaning at day 28, they were housed in groups of two to three in Macrolon cages ($40 \times 25$ cm), in temperature-controlled rooms with a standard 12:12-h L/D cycle (lights on at 0700 h). Food (Standard lab chow; RMH-B, Hope Farms) and water were available ad libitum. All experiments were performed in accordance with national laws and institutional guidelines.

### 2.2. Pharmacogenetic selected lines

Both the APO-SUS and APO-UNSUS originated from the 27th to 28th generation of selection (see Ref. [5] for the original selection procedure). Briefly, all males and females from a given generation were submitted to the apomorphine test and the mean gnawing score per gender and litter was determined. Out of the four highest APO-SUS litters, the nine highest scoring males and females were selected for the

next generation (gnawing scores >500/45 min). Likewise, out of the four lowest APO-UNSUS litters, the nine lowest scoring males and females were selected for the next generation (gnawing scores <10/45 min). This procedure led to the rapid selection of APO-SUS and APO-UNSUS animals. The apomorphine test consisted of an injection of 1.5 mg/kg, sc, and subsequent testing for stereotyped gnawing behavior in a 'gnawing' box [5]. To avoid possible effects of apomorphine on alcohol-seeking behavior, animals were not tested for their apomorphine susceptibility in this study.

### 2.3. Alcohol procedure

Each animal was individually housed and acclimatised to the drinking room for 1 week prior to the start of the experiment. Fluids (50-ml bottles) were presented to the animals in plastic cylinders mounted on top of each cage. Initially, rats were presented with tap water in both tubes for 5 days. In a two-bottle, free-choice, and continuous-access paradigm, animals were maintained on a schedule of alternate-day presentation of ethanol. Ethanol solutions were prepared by mixing 96% ethanol with tap water (v/v). On the first test day, a 2% ethanol solution was presented (in a free choice with water). Ethanol solutions were increased by 1% every alternate day, up to 10% inclusive. Water only was presented in both tubes on intervening days. The position of the tubes containing ethanol and water were altered in order to control for position bias. Fluid consumption and body weights were measured daily. Two measures of voluntary alcohol intake were determined. First, the relative intake of alcohol was calculated by counterbalancing the absolute intake for weight differences. Second, the preference of alcohol to water was calculated by dividing the alcohol intake by the total fluid intake (alcohol plus water).

### 2.4. Early postnatal manipulations

As mentioned in Section 1, the cross-fostering procedure was applied to APO-SUS animals while maternal deprivation took place in APO-UNSUS animals. Three APO-SUS groups were tested for their voluntary alcohol intake: control, in-fostered, and cross-fostered animals. All litters were culled to eight animals within 24 h after parturition, preferably, four males and four females. Control litters were left undisturbed after culling, while in-fostered and cross-fostered litters were moved to a different (nonbiological) mother immediately after culling. In-fostered litters were moved to an APO-SUS mother, cross-fostered litters to an APO-UNSUS mother. Thus, while the comparison between control and in-fostered groups gives information about the fostering effect per se, the comparison between in-fostered and cross-fostered animals is informative about the effect of the maternal environment (APO-SUS vs. APO-UNSUS). For a detailed description of the cross-fostering procedure, see Ref. [3]. Each group consisted in 9–13 animals per gender. Control animals originated from six litters, and consisted of 11 males and 13 females. In-fostered animals came from five litters, and consisted of 12 males and 11 females. Cross-fostered animals came from six litters, and consisted of 10 males and 9 females. At the start of the experiment, the average weight of the APO-SUS males varied from 265 g (cross-fostered) to 276 g (control), and 302 g (in-fostered). Control, in-fostered, and crossfosterd female APO-SUS weighed, on average, 191, 197, and 210 g, respectively. The test age of the animals varied from 9 to 12 weeks.

Similar to cross-fostering, animals in the maternal deprivation experiment were also culled to eight animals (within 24 h after parturition). Mothers were removed from the cages at postnatal day 9 (birth being postnatal day 0). Previous experiments have shown that maternal deprivation on this day produces the strongest effects in the Nijmegen Wistar population, from which APO-SUS and APO-UNSUS were selected [10]. Twenty-four hours later they were put back [8]. Litters were kept in their home cage at room temperature (22±2°C). A total of 9–14 animals of each sex were tested at the age of 9–12 weeks.

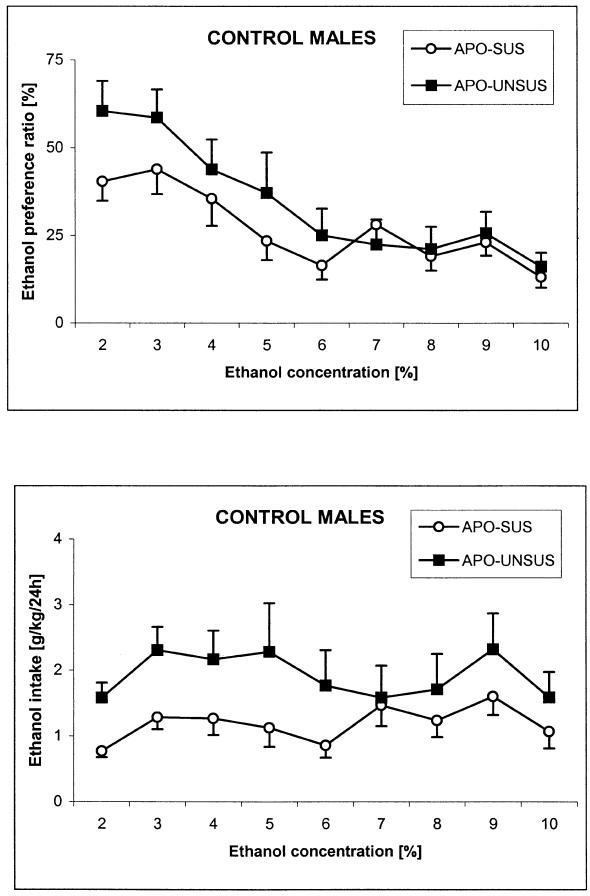


Fig. 1. Ethanol preference and intake in pharmacogenetically selected male APO-SUS and APO-UNSUS rats. Represented are means and standard errors.

Control animals originated from nine litters, and consisted of 14 males and 12 females; while maternally deprived animals came from five litters, and consisted of 11 males and 9 females. At the start of the alcohol experiment, the average weight of the APO-UNSUS males varied from 251 g (control) to 253 g (maternally deprived). Control and maternally deprived female APO-UNSUS weighed, on average, 169 and 165 g, respectively.

Not all animals from each litter (both APO-SUS and APO-UNSUS) were used for this study. Each group (defined as same sex animals that underwent the same early postnatal treatment) consisted of five or more litters. It is, therefore, unlikely that litter effects, which may be independent of maternal effects or random genetic variability within the APO-SUS and APO-UNSUS, account for possible treatment effects.

### 2.5. Statistical analysis

To test whether APO-UNSUS males consumed more alcohol than APO-SUS males (first hypothesis), we compared both 'alcohol' variables (preference and intake) using a one-way repeated ANOVA with alcohol concentration as the repeated measure, and genotype as the fixed factor. A similar ANOVA was applied to data from the APO-SUS and APO-UNSUS females. In addition, a two-way repeated measures ANOVA with alcohol concentration as the repeated measure, and genotype and sex as fixed factors was applied. Both factors had two levels (genotype: APO-SUS and APO-UNSUS; sex: male and female). Only control animals were included in this analysis.

The effects of early postnatal experiences were analyzed using a two-way repeated ANOVA with alcohol concentration as the repeated measure and treatment and sex as the fixed factors. Similar to the analysis of the control animals, two dependent variables were analyzed: preference to alcohol and the relative consumption of alcohol. Data were analyzed separately for APO-SUS and APO-UNSUS rats, as both lines received different postnatal treatments. The analysis of the APO-SUS results was performed in two steps. First, the data were compared in a two-way ANOVA with alcohol concentration as the repeated measure and treatment and sex as the fixed factors (treatment having three levels: control, in-fostered, and cross-fostered). Second, if statistically significant treatment effects occurred, a more detailed analysis was performed. On one side, the influence of the fostering per se was determined by means of comparing control to in-fostered animals. On the other side, the effect of the maternal environment was resolved through comparison of in-fostered to cross-fostered animals. The analysis of

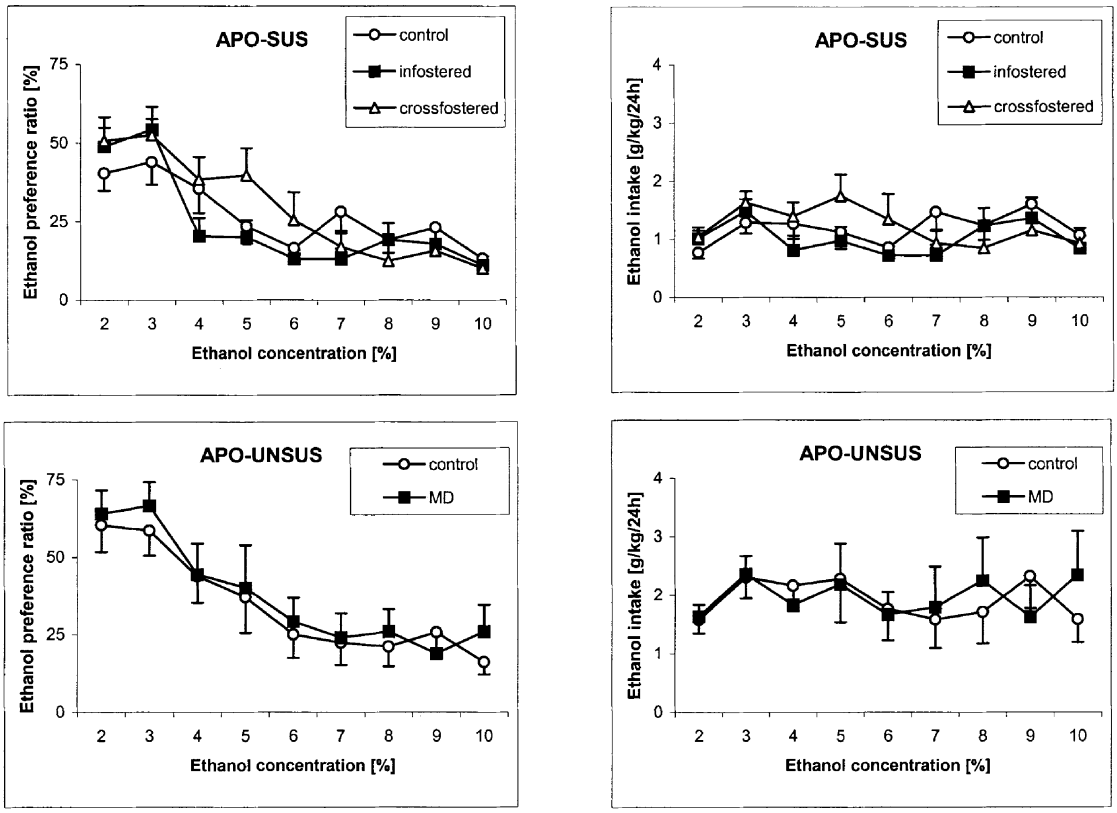


Fig. 2. Ethanol preference and intake in control, in-fostered, and cross-fostered APO-SUS males (upper panel); and control and maternally deprived APO-SUS males (lower panel). For a description of the fostering and maternal deprivation procedure (abbreviated MD), see text. Represented are means and standard errors.

the APO-UNSUS animals was more straightforward since the treatment factor consisted of just two levels: control and maternal deprivation.

## 3. Results

### 3.1. Control animals

A one-way repeated ANOVA revealed that, as expected, male APO-UNSUS rats consumed more alcohol than APO-SUS rats (preference: $F(1,23)=3.4$; $P<.05$; intake: $F(1,23)=4.2$; $P<.05$; see Fig. 1). Female APO-UNSUS animals, however, did not differ from female APO-SUS animals. Furthermore, the within-subject analysis showed that in both males and females, the preference for alcohol decreased as the alcohol concentrations increased (males: $F(8,184)=13.2$; $P<.001$; females: $F(8,184)=27.6$; $P<.001$). This effect was not found for alcohol intake and was independent of the genotype.

Surprisingly, a two-way ANOVA on all 'control' data did not find an interaction effect between sex and genotype. It did, however, reveal a genotype effect per se with APO-UNSUS animals showing a higher alcohol intake than APO-SUS animals ($F(1,46)=2.7$; $P=.05$). Also, both alcohol variables changed as the alcohol concentrations increased (preference: $F(8,368)=37.2$; $P<.001$; intake: $F(8,368)=2.8$; $P<.01$). No interaction with genotype or sex was observed.

### 3.2. Fostering in APO-SUS animals

A two-way repeated ANOVA in APO-SUS animals yielded two significant results. First, similar to control animals, both preference and intake changed as the alcohol concentrations increased (preference: $F(8,480)=62.1$; $P<.001$; intake: $F(8,480)=3.7$; $P=.001$). Second, we found a main effect for sex: females drank more than males (preference: $F(1,60)=9.7$; $P<.01$; intake: $F(1,60)=18.2$; $P<.001$). The results of the cross-fostering experiment are in the upper panel of Fig. 2. For the sake of comparison (Fig. 1), only male values are depicted.

### 3.3. Maternal deprivation in APO-UNSUS animals

Maternally deprived animals did not differ from control animals, neither for alcohol preference nor for consumption levels. This held true for both males and females (see Fig. 2, lower panel). While the relative intake remained stable, the preference for alcohol decreased as the alcohol concentrations increased ($F(8,336)=33.9$; $P<.001$).

### 3.4. All-in-all analysis

Since both postnatal manipulations did not affect ethanol self-administration, all groups were pooled to increase power and subsequently analyzed in a two-way repeated measures ANOVA with genotype and sex as fixed factors, and alcohol concentration as the repeated measure. The results confirmed what we already expected, but there was a significant interaction between genotype and sex for both alcohol variables (preference: $F(1,108)=6.7$; $P<.05$; intake: $F(1,108)=5.2$; $P<.05$). When the data were analyzed by genotype, it appeared that APO-SUS males consumed less alcohol than APO-SUS females (preference: $F(1,64)=10.3$; $P<.01$; intake: $F(1,64)=18.7$; $P<.001$), while no sex difference was found in APO-UNSUS animals. When the data were analyzed by sex, the results were similar to the outcome of the control data: only males showed a genotypic difference with APO-SUS animals consuming less alcohol than APO-UNSUS animals (preference: $F(1,56)=6.2$; $P<.05$; intake: $F(1,56)=12.2$; $P=.001$). Furthermore, both preference and intake changed as the alcohol concentrations increased (preference: $F(8,864)=93.7$; $P<.001$; intake: $F(8,864)=4.6$; $P<.001$). No interactions between alcohol concentration and genotype and/or sex were detected. Fig. 3 summarizes the results of the all-in-all analysis.

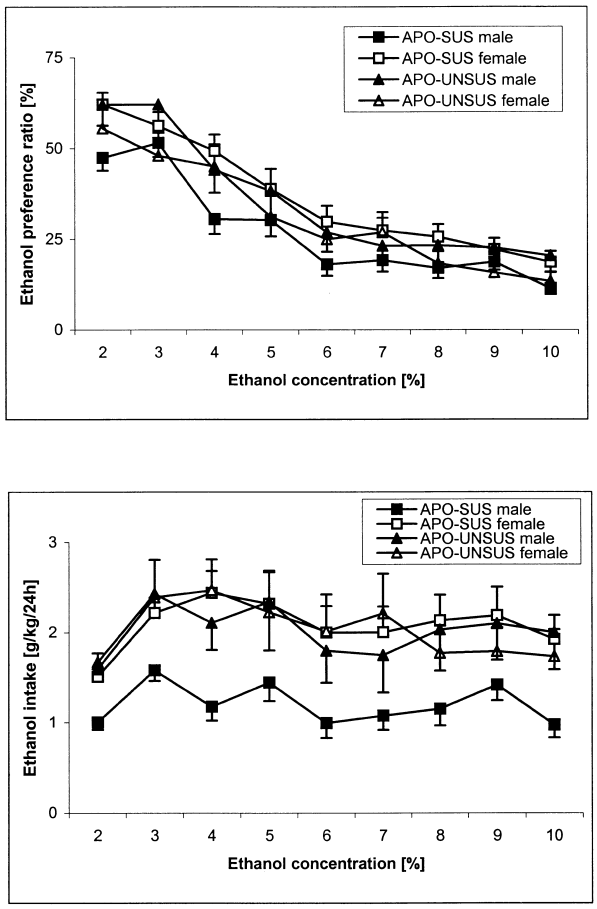


Fig. 3. Ethanol preference and intake in male and female APO-SUS and APO-UNSUS animals (mean ± standard error). Groups were pooled per genotype and sex (see 'all-in-all' analysis for more details).

## 4. Discussion

The first purpose of this study was to examine whether rat lines genetically selected for high (APO-SUS) and low (APO-UNSUS) susceptibility to the dopamine agonist apomorphine also differ in their voluntary alcohol intake. Based on previous findings in similar rat types (HR and LR, see Section 1), we hypothesized that male rats selected for low susceptibilities to apomorphine (APO-UNSUS) drink and prefer more alcohol than male rats selected for high susceptibilities to apomorphine (APO-SUS). The results support our hypothesis, and strengthen the theory that the variation in drugs of abuse, in this case ethanol self-administration, is one of the more profound differences between the two extremes of a rodent population.

The question remains why one type of animal (APO-UNSUS or LR) drinks more alcohol than the other type (APO-SUS or HR). Different levels of interpretation can be used to answer this question. For instance, a behavioral explanation for the difference in ethanol self-administration may be that APO-UNSUS rats consume more alcohol because they are more anxious than APO-SUS (unpublished results). Anxiety has been proposed to be an important factor in the initiation of alcohol consumption because it reduces the tension in anxious or stressed organisms [30]. A neurochemical explanation may be that differences in alcohol consumption are related to differences in dopaminergic transmission or dopamine receptor level. Particularly the D2 receptor subtype, which is the main target in the selection procedure for APO-SUS and APO-UNSUS, has been the object of many studies. For instance, studies in other selected lines have demonstrated that the predisposition to drink excessive amounts of alcohol is associated with low dopamine D2 receptor densities and low mesolimbic dopaminergic activities [21,31]. Although APO-UNSUS males have lower D2 receptor densities in the striatal projection area of the substantia nigra and in the tubero-infindibular system than APO-SUS males, D2 receptor levels in the mesolimbic pathway are similar [27]. An interesting finding in this respect is the phenomenon that wild-type and heterozygous knockouts for the D2 receptor drink similar amounts of alcohol, suggesting that merely a large reduction in D2 receptors affects ethanol self-administration [23]. An alternative interpretation for the difference in alcohol intake between APO-SUS and APO-UNSUS males is that these lines vary in taste, rather than the post-ingestive effects of alcohol. Generally, alcohol consumption in these lines was low, suggesting that blood alcohol levels never reached a pharmacologically effective dose range, and could, therefore, never be reinforcing. In fact, APO-SUS males avoided the ascending series of alcohol solutions before APO-UNSUS males did, implying a difference in the response to the aversive properties of increasing alcohol concentrations. This finding is not consistent with previous experiments in HR and LR rats [13]. HR and LR rats were found to be either equally sensitive to the aversive properties of a 0.01% quinine solution, or differed in a direction opposite to the current results, with LR rats showing greater sensitivity to the aversive qualities of a 15% sucrose solution. Theoretically, it is possible that the difference in alcohol intake between the APO-SUS and APO-UNSUS lines is due to the random fixation of alleles (genetic drift) rather than to the alleles affected by selective breeding. In other words, differences in dopaminergic activities may be spuriously related to variation in voluntary alcohol intake. This is unlikely, however, given the differences in alcohol consumption between the comparable HR and LR rats.

The second conclusion from this study is that, in contrast to our expectations, neither maternal deprivation nor cross-fostering influence the voluntary alcohol intake in these selection lines. As already mentioned in Section 1, early postnatal manipulations have rarely been used in studies on alcohol intake. To our knowledge, cross-fostering has only been used once to study the voluntary alcohol consumption in rodents [25]. It appeared that when mice from an alcohol avoiding strain (DBA) were raised by mothers from an alcohol preferring strain (C57BL), they drank twice as much alcohol as did nonfostered DBA mice. The reciprocal procedure showed no effects: C57BL mice raised by DBA mother consumed similar amounts of alcohol as did nonfostered C57BL mice. In humans, however, the equivalent of cross-fostering, the adoption design, is frequently applied. In adoption studies, traits in adoptees are compared with those in both their biological and adoptive relatives. One of the more influential studies on adoption data extended and reanalyzed existing data, and by using sophisticated multivariate statistics, Cloninger et al. [4] proposed the existence of two subtypes of alcoholism. Type I occurs both in men and women, and is characterized by mild adult-onset abuse and influenced by both genetic and environmental factors. Type II, on the other hand, is limited to males, marked by teenage onset and has a strong genetic component.

So far only two studies have systematically examined the effect of maternal deprivation on voluntary alcohol intake in rodents and both with conflicting results. The results of Hilakivi Clarke et al. [15] are in agreement with our findings. They did not find an effect on voluntary alcohol intake in animals separated from their mother for 1 h daily from day 5 to day 20, inclusive. Rockman et al. [26], however, observed that rats that were weaned early at day 16 consumed more alcohol than rats weaned later in life. One major drawback in comparing rodent studies on the effects of maternal deprivation is methodology, particularly, the timing and duration of the maternal deprivation. Nevertheless, a recent study on monkeys underlined the importance of maternal separation on alcohol consumption. Rhesus macaques separated from their mothers at birth drink more alcohol at adult age than those that remain with their mothers [11]. They also showed higher cortisol levels in response to stress, which has been suggested to be a marker for alcohol abuse and dependence, but which does

not fit the data on our selection lines. That is, APO-SUS male rats exhibit higher and prolonged plasma ACTH and corticosterone than APO-UNSUS rats after exposure to a novel environment [28].

Early postnatal events are influential factors in the ontogeny of other features of the two fundamentally different rat characters. For instance, the genetic predisposition to periodontitis, which is a destructive inflammatory disorder caused by inappropiate immune reactions, can be completely reversed by maternal deprivation and cross-fostering. Normally, APO-SUS animals develop more fiber and bone loss, i.e., periodontal breakdown, than APO-UNSUS animals. However, cross-fostering reverses an APO-SUS animal into an APO-UNSUS, while, conversely, maternal deprivation changes an APO-UNSUS into an APO-SUS [29].

Female APO-SUS and APO-UNSUS drink similar amounts of alcohol, a result not expected from previous experiments on other correlates in these lines [29]. Together with the absence of effects of early postnatal manipulations, these findings suggest that neither manipulations during the early postnatal period (i.e., cross-fostering and maternal deprivation) nor genetic influence (i.e., selective breeding for high and low dopaminergic activity) act upon voluntary alcohol intake in the female Nijmegen Wistar population. We can only speculate on which factors affect the individual variation in alcohol-seeking behavior in females of this outbred rat population. It seems unlikely that within-group differences in hormonal levels in females increase the variation in the trait under investigation to such an extent that between-group differences are below detection. After all, measuring ethanol self-administration takes 18 days (more or less four estrous cycles) and individual differences will therefore be less affected by hormonal changes. Hence, a different explanation seems necessary to explain individual variation. Genetically, allelic variation in genes other than those affecting dopaminergic activity, e.g., serotonergic or noradrenergic activity, might affect voluntary alcohol intake. Our findings suggest, however, that this genetic variation is equal across the APO-SUS and APO-UNSUS rat lines. Another explanation is the importance of time points other than the early postnatal period. The early postpubertal period, for example, has been shown to be critical in the development of alcohol consumption in females [19]. Variation in estrogen and progesterone among females may lead to qualitative and quantitative differences in steroid-responsive receptors, which, in turn, may modulate the variation in voluntary alcohol intake.

The general finding that female rats consume more alcohol than male rats (see, among others, Ref. [20]) is only observed in APO-SUS animals. Various hypotheses have been put forward to explain gender differences in alcohol intake in rats. Hormonal variation might be responsible [1,19], but differences in the brain are probably the most important source of variation in determining sex differences in ethanol self-administration [18]. Particularly, the release of dopamine in the nucleus accumbens has been associated with variation in alcohol consumption in male and female rats [2]. However, the problem remains why this sex difference in ethanol self-administration in rodents is only apparent in one line. In fact, a meta-analysis of the different experiments in this study, with only genotype and sex as discriminative factors, confirmed and emphasized what is already visible in the control lines: the APO-SUS males are the exception. They consume less alcohol than the other three groups. An explanation might be sex-specific genetic differences. In this regard, a recent study on recombinant inbred mice is noteworthy, where female-specific quantitative trait loci were observed [12]. Clearly, further research is needed to elucidate this strain- and sex-specific difference in APO-SUS and APO-UNSUS.

In conclusion, male rats genetically selected for low susceptibilities to apomorphine (APO-UNSUS) consume more alcohol than male rats genetically selected for high susceptibilities to apomorphine (APO-SUS). Neither cross-fostering in APO-SUS, nor maternal deprivation in APO-UNSUS animals, affects this difference in drinking behavior. Sex differences are restricted to APO-SUS animals, where females drink more than males.

## Acknowledgments

FS was supported by a PULS Grant (48.001) from the Earth and Life Sciences Foundation (ALW), which is subsidized by the Netherlands Organization for Scientific Research (NWO). MH was also supported by ALW (805-46-011).

## References

[1] Almeida OFX, Shoaib M, Deicke J, Fischer D, Darwish MH, Patchev VK. Gender differences in ethanol preference and ingestation in rats. J Clin Invest 1998;101:2677–85.

[2] Blanchard BA, Glick SD. Sex differences in mesolimbic dopamine responses to ethanol and relationship to ethanol intake in rats. Recent Dev Alcohol 1995;12:231–41.

[3] Carlier M, Nosten-Bertrand M, Michard-Vanhee C. Separating genetic effects from maternal environmental effects. In: Goldowitz D, Wahlsten D, Wimer R, editors. Techniques for the genetic analysis of brain and behavior: focus on the mouse. Techniques in the behavioral and neural sciences, vol. 8. Amsterdam: Elsevier, 1992. pp. 111–25.

[4] Cloninger CR, Bohman M, Sigvardsson S. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. Arch Gen Psychiatry 1981;38:861–8.

[5] Cools AR, Brachten R, Heeren D, Willemen A, Ellenbroek B. Search after neurobiological profile of individual-specific features of Wistar rats. Brain Res Bull 1990;24:49–69.

[6] Cools AR, Gingras MA. Nijmegen high and low responders to novelty: a new tool in the search after the neurobiology of drug abuse liability. Pharmacol, Biochem Behav 1998;60:151–9.

[7] El Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T, Liu C, Sibley DR, Westphal H, O'Dowd BF. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. Eur J Pharmacol 1998;353:149–58.

[8] Ellenbroek BA, Cools AR. Maternal separation reduces latent inhibition in the conditioned taste aversion paradigm. Neurosci Res Commun 1995;17:27–33.
[9] Ellenbroek BA, Sluyter F, Cools AR. The role of genetic and early environmental factors in determining apomorphine susceptibility. Psychopharmacology 2000;148:124–31.
[10] Ellenbroek BA, van den Kroonenberg PTJM, Cools AR. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr Res 1998;30:251–60.
[11] Fahlke C, Lorenz JG, Long J, Champoux M, Suomi SJ, Higley JD. Rearing experiences and stress-induced plasma cortisol as early risk factors for excessive alcohol consumption in nonhuman primates. Alcohol. Clin Exp Res 2000;24:644–50.
[12] Gill K, Desaulniers N, Desjardins P, Lake K. Alcohol preference in AXB/BXA recombinant inbred mice: gender differences and gender-specific quantitative trait loci. Mamm Genome 1998;9:929–35.
[13] Gingras MA. Individual differences in behavioral responses to drugs of abuse. PhD thesis. University of Nijmegen, Netherlands, 1997.
[14] Gingras MA, Cools AR. Differential ethanol intake in high and low responders to novelty. Behav Pharmacol 1995;6:718–23.
[15] Hilakivi Clarke LA, Turkka J, Lister RG, Linnoila M. Effects of early postnatal handling on brain beta-adrenoceptors and behavior in tests related to stress. Brain Res 1991;542:286–92.
[16] Kaij L. Alcoholism in twins. Stockholm: Almqvist & Wiksell, 1960.
[17] Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci 1992;13:177–84.
[18] Lancaster FE. Gender differences in the brain: implications of the study of human alcoholism. Alcohol. Clin Exp Res 1994;18:740–6.
[19] Lancaster FE, Brown TD, Coker KL, Elliott JA, Wren SB. Sex differences in alcohol preference and drinking patterns emerge during the early postpuberal period in Sprague–Dawley rats. Alcohol. Clin Exp Res 1996;20:1043–9.
[20] Lancaster FE, Spiegel KS. Sex differences in pattern of drinking. Alcohol 1992;9:415–20.
[21] McBride WJ, Murphy JM, Gatto GJ, Levy AD, Yoshimoto K, Lumeng L, Li TK. CNS mechanisms of alcohol self-administration. Alcohol Alcohol 1993;(Suppl 2):463–7.
[22] McClearn GE, Rodgers DA. Differences in alcohol preference among inbred strains of mice. Q J Stud Alcohol 1959;20:691–5.
[23] Phillips TJ, Brown KJ, Burkhart Kasch S, Wenger CD, Kelly MA, Rubinstein M, Grandy DK, Low MJ. Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. Nat Neurosci 1998;1:610–5.
[24] Phillips TJ, Crabbe JC. Behavioral studies of genetic differences in alcohol action. In: Crabbe JC, Harris RA, editors. The genetic basis of alcohol and drug actions. New York: Plenum, 1991. pp. 25–105.
[25] Randall CL, Lester D. Cross-fostering of DBA and C57Bl mice. Increase in voluntary consumption of alcohol by DBA weanlings. J Stud Alcohol 1975;36:973–80.
[26] Rockman GE, Hall A, Markert L, Glavin GB. Early weaning effects on voluntary ethanol consumption and stress responsivity in rats. Physiol Behav 1987;40:673–6.
[27] Rots NY, Cools AR, Berod A, Voorn P, Rostene W, de Kloet ER. Rats bred for enhanced apomorphine susceptibility have elevated tyrosine hydroxylase mRNA and dopamine D2-receptor binding sites in nigrostriatal and tuberoinfundibular dopamine systems. Brain Res 1996;710:189–96.
[28] Rots NY, Cools AR, Oitzl MS, de Jong J, Sutanto W, de Kloet ER. Divergent prolactin and pituitary–adrenal activity in rats selectively bred for different dopamine responsiveness. Endocrinology 1996;137:1678–86.
[29] Sluyter F, Hof M, Breivik T, Cools AR. On the sensitivity to periodontitis in Wistar rats: genetic and early postnatal effects. Soc Neurosci Abstr 1999;29:1127.
[30] Spanagel R, Montkowski A, Allingham K, Stöhr T, Shoaib M, Holsboer F, Landgraf R. Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. Psychopharmacology 1995;122:369–73.
[31] Stefanini E, Frau M, Garau MG, Garau B, Fadda F, Gessa GL. Alcohol-preferring rats have fewer dopamine D2 receptors in the limbic system. Alcohol Alcohol 1992;27:127–30.