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Sex-Dependent Modulation of Ethanol Consumption in Vesicular Monoamine Transporter 2 (VMAT2) and Dopamine Transporter (DAT) Knockout Mice

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Several lines of evidence suggest that monoaminergic systems, especially dopaminergic and serotoninergic systems, modulate ethanol consumption. Humans display significant differences in expression of the vesicular and plasma membrane monoamine transporters important for monoaminergic functions, including the vesicular monoamine transporter (VMAT2, SLC18A2) and dopamine transporter (DAT, SLC6A3). In addition, many ethanol effects differ by sex in both humans and animal models. Therefore, ethanol consumption and preference were compared in male and female wild-type mice, and knockout (KO) mice with deletions of genes for DAT and VMAT2. Voluntary ethanol (2–32% v/v) and water consumption were compared in two-bottle preference tests in wild-type (+/+) *vs* heterozygous VMAT2 KO mice (+/−) and in wild-type (+/+) *vs* heterozygous (+/−) or homozygous (−/−) DAT KO mice. Deletions of either the DAT or VMAT2 genes increased ethanol consumption in male KO mice, although these effects were highly dependent on ethanol concentration, while female DAT KO mice had higher ethanol preferences. Thus, lifetime reductions in the expression of either DAT or VMAT2 increase ethanol consumption, dependent on sex.
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INTRODUCTION

Ethanol consumption has monoaminergic as well as nonmonoaminergic components (Gianoulakis, 2001). Dopamine and serotonin systems have been especially implicated in ethanol consumption (for review see Nutt, 1999; Grace, 2000; Li, 2000). Many features of human and rodent ethanol consumption also display substantial sex dependence, although interactions between sex and monoamine effects have been studied only rarely.

A dopaminergic role in the effects of alcohol is supported by evidence from a variety of fields. Ethanol stimulates the firing of dopaminergic neurons (Mereu *et al*, 1984; Mereu and Gessa, 1984; Brodie *et al*, 1990, 1999), and increases extracellular levels of dopamine in the nucleus accumbens (Wozniak *et al*, 1991; Yoshimoto *et al*, 1992a, b; Kiianmaa *et al*, 1995). A specific role for dopamine in ethanol reward is supported by data showing that dopaminergic agents and lesions of dopamine systems modify ethanol self-administration (Levy *et al*, 1991; Dyr *et al*, 1993; Russell *et al*, 1996; Hodge *et al*, 1997; Ikemoto *et al*, 1997) and by results documenting that ethanol is directly self-administered into the ventral tegmental area (Gatto *et al*, 1994). Animal models also support a role for dopaminergic differences in the genetic determination of ethanol reward. Rats bred for ethanol preference display dopamine receptor (Stefanini *et al*, 1992; McBride *et al*, 1993) and forebrain dopamine levels different from ethanol nonpreferring rats (Zhou *et al*, 1995), and dopamine receptor gene knockouts (KOs) also alter ethanol self-administration (Crabbe *et al*, 1996; Risinger *et al*, 1996, 1999, 2000; Rubinstein *et al*, 1997; El-Ghundi *et al*, 1998; Phillips *et al*, 1998).

Serotonin function has also been associated with ethanol consumption and alcoholism (for reviews see Lovinger, 1999; Hoffman *et al*, 2001; Myrick *et al*, 2001; Weiss *et al*, 2001). Reduced serotonin function has been especially postulated to predispose to alcoholism (Myers and Melchior, 1977). Reduced cerebrospinal fluid levels of the serotonin metabolite 5-HIAA are observed in some alcoholics (Linnoila *et al*, 1983), and are associated with increased ethanol consumption in rhesus monkeys (Higley *et al*, 1996). Ethanol-preferring rats display alterations in tissue serotonin levels (McBride *et al*, 1990, 1991; Aulakh *et al*, 1994; Zhou *et al*, 1994), responses to serotoninergic agents (Gudelsky *et al*, 1985; Aulakh *et al*, 1988a, b, 1992, 1994; Wang *et al*, 1988), serotonin reuptake (Arora *et al*,

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1983; Hulihan-Giblin *et al*, 1993; Chen and Lawrence, 2000), and serotonin receptor densities (Hulihan-Giblin *et al*, 1992, 1993; Wong *et al*, 1993; McBride *et al*, 1994, 1997; Chen and Lawrence, 2000). A variety of serotonergic agents can reduce voluntary ethanol consumption in animal models and in humans (for a review, see Lovinger, 1999; Myrick *et al*, 2001), and serotonin receptor gene KOs also affect ethanol self-administration (Crabbe *et al*, 1996; Risinger *et al*, 1996, 1999, 2000; Rubinstein *et al*, 1997; El-Ghundi *et al*, 1998; Phillips *et al*, 1998).

Dopamine and 5-HT involvement in the rewarding effects of ethanol need not be independent however. Ethanol elevates both serotonin and dopamine concentrations when administered into the VTA (Yan *et al*, 1996), and 5-HT3 receptor antagonists attenuate the dopamine release in the nucleus accumbens that is triggered by systemic ethanol (Wozniak *et al*, 1990). Vesicular monoamine transporter 2 (VMAT2) (Erickson *et al*, 1996; Takahashi and Uhl, 1997) mediates the vesicular storage of both dopamine and serotonin in synaptic vesicles (Gasnier, 2000; Uhl *et al*, 2000), and is therefore well positioned to regulate both dopaminergic and serotonergic neurotransmission, and perhaps alcohol consumption as well. DAT (Donovan *et al*, 1995) is similarly positioned to influence dopamine function since it mediates much of the clearance of released dopamine from the synaptic cleft. While effects of altering these genes' expression on psychostimulant reward and locomotion have been examined, there are no current data describing the effects of VMAT2 or DAT KOs on ethanol consumption.

There is substantial evidence for sex-dependent differences in alcoholism and in animal models of alcoholism (for a review see Lancaster, 1994, 1995). Human alcoholism subtypes (Cloninger, 1987) may be differentially represented in males and females (Gilligan *et al*, 1987), and there is evidence for differential heritability of alcohol dependence in men and women in some (eg Han *et al*, 1999), but not all, studies (Heath *et al*, 1997). Furthermore, ethanol consumption is reduced by μ opiate receptor (MOR) gene KO in a sex-dependent fashion (Hall *et al*, 2001), so sex dependence might be expected in other alcohol-related genes as well. Despite this evidence, most human studies of dopaminergic and serotonergic gene polymorphisms have examined only males (Dobashi *et al*, 1997; Sander *et al*, 1997a; Iwata *et al*, 1998; Lappalainen *et al*, 1998, 1999; Nielsen *et al*, 1998; Sander *et al*, 1999; Schuckit *et al*, 1999), studied subject pools that are largely male (Gelernter *et al*, 1991, 1997; Higuchi *et al*, 1994; Muramatsu and Higuchi, 1995; Sander *et al*, 1997a, b; Ueno *et al*, 1999; Matsushita *et al*, 2001), omitted statements about the sex of the subjects tested (Pesonen *et al*, 1998; Sander *et al*, 1998; Gelernter and Kranzler, 1999; Blomqvist *et al*, 2000; Vandenbergh *et al*, 2000), or examined pooled data with sex assumed to be irrelevant (Goldman *et al*, 1997, 1998; Parsian and Zhang, 1997; Franke *et al*, 1999; Laine *et al*, 2001a, b). When a recent study examined male and female alcoholics separately, it found sex-dependent interactions between X chromosome-linked monoamine oxidase A (MAOA) alleles and alcoholism subtypes (Schmidt *et al*, 2000).

Based on these considerations, we now report sex-specific assessments of ethanol consumption in VMAT2 (+/+ and +/−) and DAT (+/+, +/−, and −/−) KO strains (Takahashi *et al*, 1997; Sara *et al*, 1998). Data from heterozygous mice are especially interesting since such animals provide approximate models for the magnitude of human inter-individual differences in the levels of expression of these two genes (see Uhl, 1998).

MATERIALS AND METHODS

Subjects: VMAT2 and DAT KO Mice

Mice were bred by random heterozygote crosses of mice developed in our laboratory maintained on a mixed C57/129sv background: VMAT +/+ and +/− (Takahashi *et al*, 1997); DAT +/+, +/−, and −/− (Sora *et al*, 1998). Most VMAT2 −/− mice die by the third postnatal day. The mice used in these experiments were from greater than the tenth generation of mice in these lines. All generations were produced from crosses of heterozygous mice. Mice were weaned at 21 days of age, and housed with same-sex littermates for the duration of the experiments. Standard colony conditions were used: 24°C, 50% relative humidity, and *ad libitum* food and water according to AALAC guidelines. Experimentation began at between 8 and 12 weeks of age, at which point mice were housed singly.

At weaning, 0.5 cm tail samples were taken for genotyping by PCR. Tail samples were incubated overnight at 55°C in tail buffer (50 mM Tris, pH 8.0; 100 mM EDTA; 100 mM NaCl; 1% SDS) containing Protease K (10 mg/ml). Supernatants were removed and lysis buffer added (0.32 M sucrose; 10 mM Tris, pH 7.5; 5 mM MgCl; 1% Triton X-100). After centrifugation the supernatant was removed and the tail DNA solution was used for PCR using PCR buffer (Lambda Biotech), 1 mM dNTP mix (Lambda Biotech), 25 mM $MgCl_2$ (Lambda Biotech; final concentration of 4 mM), and 3.1 U/tube Tsg DNA Polymerase (Lambda Biotech, 5 U/μl).

Oligonucleotides (10 μM) for VMAT2 included a forward primer located outside the deleted region (5′GCT TAC CTC GTG GGC ATG GTG 3′), a reverse primer for the VMAT2 gene located in the region of the gene which is deleted in the KO (5′ GTC CCC AGT TTA TGT AGC ATT G 3′), and a reverse primer for the NEO gene (5′ TCG ACG TTG TCA CTG AAG CGG 3′). Amplimers from wild-type DNA were 1000 bp and amplimers from KOs were 700 bp.

For DAT genotyping, oligonucleotides (10 μM) included a forward primer located outside the deleted region (5′ GTG CCT AAG GTG CTC ACG GAG 3′), a reverse primer for the DAT gene located in the region which is deleted in the KO (5′ CAC AGC TCT GGC AGG TCT CAG 3′), and a reverse primer for the NEO gene (5′ GCC TCT GTC CGC AGT TCA TTC AG 3′). Amplimers from wild-type mice were 640 bp and from KOs were 900 bp.

Experiment 1. Voluntary Ethanol Consumption in VMAT2 KO Mice

Experimentally naïve male and female littermates ($N = 15$ per genotype; >10th generation) were housed singly beginning 1 week prior to the experiments. Water, food, and ethanol consumption were monitored in home cages. Initially, only food and water were available to determine

baseline consumption. Subsequently, the subjects were given access to food, water, and ethanol in a standard two-bottle home-cage consumption paradigm. Fluids were made available in 50 ml polypropylene centrifuge tubes capped with rubber stoppers and standard sipper tubes (control experiments, data not shown, showed that spillage and evaporation from these tubes was less than 0.1 ml per measurement interval). Mice were weighed weekly, fluid bottles and food were weighed every 2–3 days, and consumption was calculated in g/kg/day, ml/kg/day, and g/kg body weight/day, respectively, for food, water, and ethanol. The initial ethanol concentration was 2% and concentrations were increased every 2–3 days in the following progression: 2, 4, 8, 12, 16, 24, and 32%. The positions of the bottles were switched each time the bottles were changed.

Experiment 2. Voluntary Ethanol Consumption in DAT KO Mice

Experimentally naïve male and female littermates ($N = 9$–11 per genotype; >10th generation) were studied as described previously. However, 11 of 20 DAT −/− mice died within 2 days of single housing, while none of the DAT +/+ or DAT +/− mice died. DAT −/− mice stopped drinking and eating (eg spontaneous adipsia and aphagia) under these conditions. Similar mortality, which we have previously observed in DAT −/− mice in our breeding facility (unpublished findings), may also be the result of this spontaneous adipsia/aphagia.

Data Analysis

Data were analyzed by ANOVA with the between-subjects measures of GENOTYPE and SEX. Subsequently, because ethanol consumption differed between male and female mice, and significant GENOTYPE × SEX interactions were observed in some cases, separate ANOVAs were performed on data from males and females for all data. Consumption data were analyzed as grams of ethanol per kilogram body weight per day (g/kg/day). The within-subjects factor of CONCENTRATION was used for these data. Although food and water consumption was measured throughout, only the baseline data are presented, expressed in g/kg/day and ml/kg/day, respectively. *Post hoc* analyses were performed using Scheffe's *post hoc* comparisons.

RESULTS

Experiment 1. Voluntary Ethanol Consumption in VMAT2 +/+ and +/− Mice

Baseline food and water consumption. Male mice weighed more than female mice (Table 1: $F[1,56] = 37.5$, $p < 0.0001$), but there was no significant effect of GENOTYPE ($F[1,56] = 0.7$, NS). When food consumption was expressed as g/kg/day it was found that male VMAT2 +/− mice ate more than female VMAT2 +/− mice, but no such difference was observed in VMAT2 +/+ mice (Table 1: GENOTYPE × SEX $F[1,56] = 6.0$, $p < 0.02$). Male VMAT2 +/− consumed more food than male VMAT2 +/+ mice, and female VMAT2 +/− mice consumed less food than femaleVMAT2 +/+ mice, but neither of these effects was statistically significant according to *post hoc* analysis. An identical GENOTYPE × SEX effect was found for water consumption (Table 1: GENOTYPE × SEX $F[1,56] = 6.8$, $p < 0.02$).

Table 1 Baseline measures in VMAT2 KO mice

	VMAT2 +/+		VMAT2 +/−	
	Male	**Female**	**Male**	**Female**
Wt (g)	42.9 ± 2.3*	28.8 ± 1.0	39.1 ± 2.7*	29.5 ± 1.0
Food (g/kg/day)	3.7 ± 0.2	3.6 ± 0.2	4.5 ± 0.3*	3.2 ± 0.2
Water (ml/kg/day)	3.0 ± 0.4	3.5 ± 0.4	4.5 ± 0.5	2.9 ± 0.3

Data: weight (g), food consumption (g/kg/day), and water consumption (ml/kg/day). The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post hoc* comparisons, male *vs* female, $p < 0.05$.

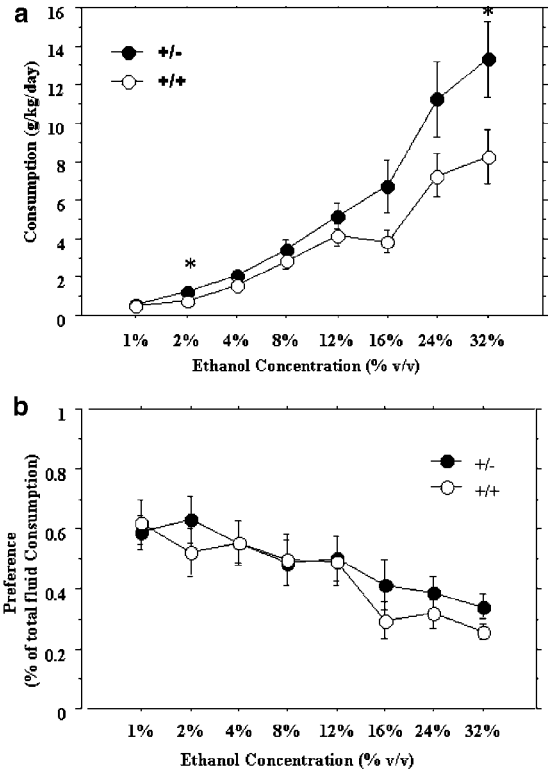

Figure 1 Male VMAT2 KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for male +/+, and +/− VMAT2 KO mice. The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post hoc* comparisons, +/+ *vs* +/−, $p < 0.05$.

Ethanol consumption and preference. Female mice consumed more ethanol than male mice (Figures 1a and 2a). An overall ANOVA for ethanol consumption, including SEX as a factor, revealed significant effects of SEX ($F[1,56] = 11.2$, $p < 0.002$) and CONCENTRATION ($F[7,392] = 101.5$, $p < 0.0001$). Ethanol preference was also affected by ethanol CONCENTRATION (Figures 1b and 2b; $F[1,56] = 11.8$, $p < 0.0001$), but not by any other factors.

When the data were analyzed separately by SEX, ANOVA of the data from male mice revealed more consumption of higher-concentration ethanol solutions in VMAT2 +/− than in VMAT2 +/+ mice (Figure 1a; GENOTYPE

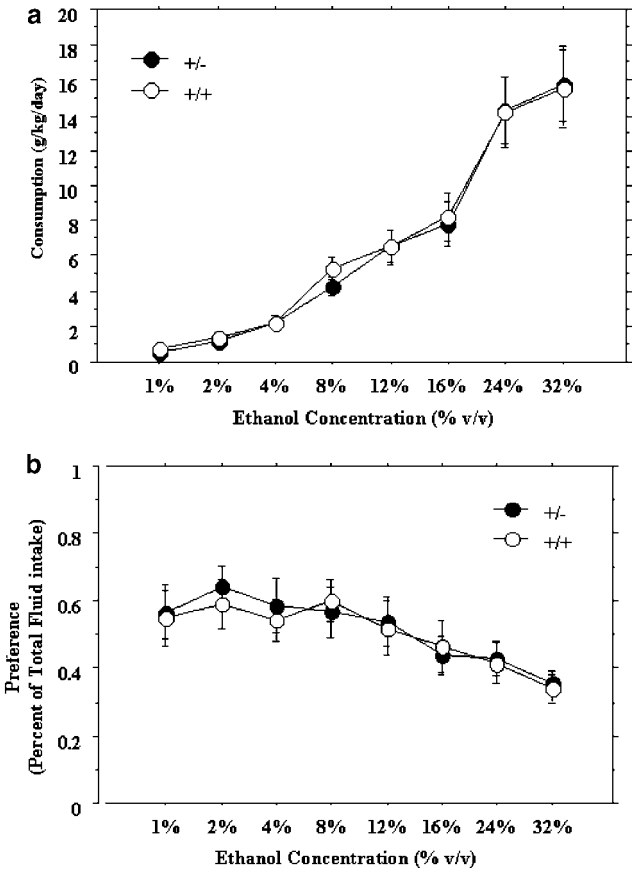

Figure 2 Female VMAT2 KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for female +/+, and +/− VMAT2 KO mice. The data are expressed as mean ± SEM.

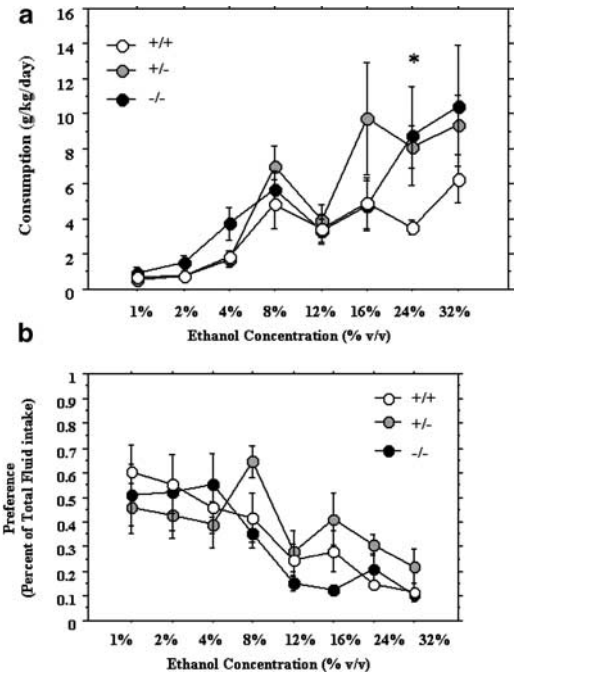

Figure 3 Male DAT KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for male +/+, +/−, and −/− DAT KO mice. The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post hoc* comparisons, +/+ *vs* +/−, $p<0.05$.

$F[1,28]=5.6$, $p<0.03$; GENOTYPE × CONCENTRATION $F[7,196]=2.5$, $p<0.02$). There was no difference in ethanol consumption between female VMAT2 +/+ and +/− mice (Figure 2a; GENOTYPE $F[1,28]=0.0$, NS; GENOTYPE × CONCENTRATION $F[7,196]=0.1$, NS). There were no differences between genotypes in ethanol preference for males (Figure 2a; GENOTYPE $F[1,28]=0.5$, NS) or females (Figure 2b; GENOTYPE $F[1,28]=0.5$, NS).

Experiment 2. Voluntary Ethanol Consumption in DAT KO Mice

Baseline food and water consumption. Male mice weighed more than female mice (Table 2: $F[1,45]=55.4$, $p<0.0001$), independent of genotype. DAT −/− mice weighed less than either +/+ or +/− mice (Table 2: GENOTYPE $F[2,45]=10.9$, $p<0.001$), as previously reported (Sora *et al*, 1998). Overall, males consumed more food than females (Table 2: SEX $F[1,45]=5.2$, $p<0.03$), but this difference was not observed in DAT −/− mice (significant *post hoc* Scheffe's comparison between males and females in DAT +/+ and +/− mice, but not DAT −/− mice). Despite these differences in food consumption, there was no effect of either GENOTYPE or SEX on water consumption (Table 2: GENOTYPE $F[2,45]=0.7$, NS; SEX $F[1,45]=0.6$, NS).

Ethanol consumption and preference

Overall, ANOVA for ethanol consumption, including SEX as a factor, revealed significant effects of CONCENTRATION (Figures 3a and 4a; $F[7,315]=27.9$, $p<0.0001$), SEX ($F[1,45]=35.9$, $p<0.0001$), and a GENOTYPE × SEX interaction ($F[2,45]=3.2$, $p<0.05$). Overall, ethanol preference was affected by CONCENTRATION (Figures 3b and 4b; $F[7,315]=10.5$, $p<0.0001$) and by GENOTYPE

Table 2 Baseline measures in DAT KO mice

	DAT +/+		DAT +/−		DAT −/−	
	Male	Female	Male	Female	Male	Female
Wt (g)	39.2 ± 2.2*	24.4 ± 0.7	39.5 ± 2.3*	24.2 ± 0.7	26.3 ± 4.4*,‡	17.7 ± 0.9‡
Food (g/kg/day)	4.0 ± 0.5*	2.9 ± 0.1	4.5 ± 0.6*	2.8 ± 0.1	2.8 ± 0.5	2.9 ± 0.5
Water (ml/kg/day)	3.7 ± 0.3	4.5 ± 0.5	4.1 ± 0.3	4.2 ± 0.7	3.7 ± 0.2	3.3 ± 0.3

Data weight (g), food consumption (g/kg/day), and water consumption (ml/kg/day). The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post hoc* comparisons, male *vs* female, $p<0.05$. ‡Significant *post hoc* difference using Scheffe's comparison, +/+ *vs* −/−, $p<0.05$.

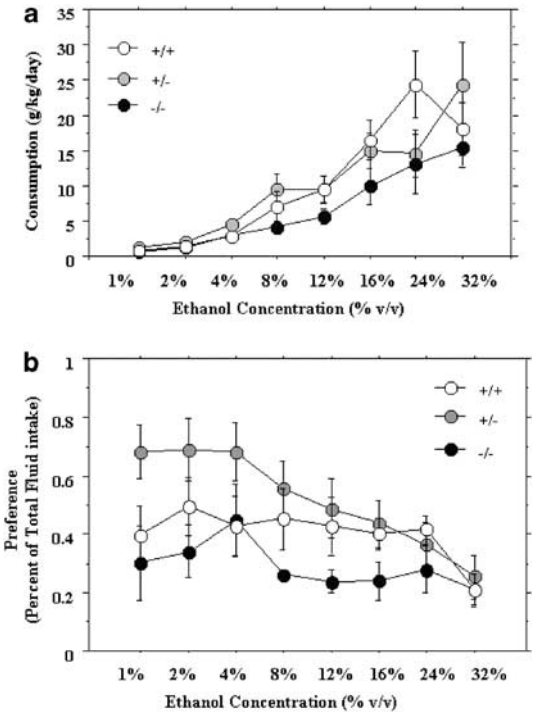

Figure 4 Female DAT KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for female +/+, +/−, and −/− DAT KO mice. The data are expressed as mean ± SEM.

($F[2,45] = 4.0$, $p < 0.03$). As before, female mice consumed more ethanol than male mice.

When the data from each SEX were analyzed separately, it was found that male DAT +/− and −/− mice consumed more ethanol than DAT +/+ mice, particularly at high ethanol concentrations (Figure 3a; GENOTYPE $F[2,23] = 3.6$, $p < 0.05$). The genotype difference was only significant, by *post hoc* Scheffe's comparisons, at the high ethanol concentrations. Ethanol preference did not differ significantly in male mice based on GENOTYPE (Figure 3b; $F[2,23] = 0.6$, NS) or GENOTYPE × CONCENTRATION interaction ($F[7,161] = 1.1$, NS).

There were no differences in ethanol consumption between female DAT +/+ mice and female DAT +/− or −/− mice (Figure 4a; GENOTYPE $F[2,22] = 2.5$, NS; GENOTYPE × CONCENTRATION $F[7,154] = 1.0$, NS), although there was a trend for female DAT +/− to consume more, and female DAT −/− less, ethanol than female DAT +/+ mice at lower ethanol concentrations. Preference assessments did reveal (Figure 4b) significant effects of both CONCENTRATION ($F[7,154] = 3.8$, $p < 0.001$) and GENOTYPE ($F[2,22] = 4.1$, $p < 0.04$). Female DAT +/− mice displayed ethanol preferences greater than those of both +/+ and −/− mice, the latter also having trends toward reduced ethanol preference.

DISCUSSION

Deletions of either the DAT or the VMAT2 genes increased ethanol consumption in male but not female mice, although female DAT +/− mice displayed higher ethanol preferences and there was a trend for female DAT −/− mice to consume less ethanol and to have lower preferences. These observations add to the wealth of evidence that these neurotransmitter systems modulate ethanol consumption, and also provide working hypotheses about the ways in which human interindividual differences in the expression of these genes could affect human ethanol consumption.

A recent study in DAT KO mice (Savelieva *et al*, 2002) found decreased consumption in female DAT KO mice (where a trend toward reduced consumption was observed in the present study), while the increased consumption in male DAT KO mice observed in the present study was paralleled (in absolute terms, although the differences were not statistically significant) in the Savelieva *et al* (2002) study. Despite the apparent discrepancies between the two reports, the overall pattern of effects is strikingly similar even though the magnitude of differences in males and females is reversed in the two studies. The reasons for this divergence can only be speculated at, but might denote differences in genetic background as the two strains were developed independently but certainly are consistent with the view that there are multiple genetic and nongenetic determinants of ethanol consumption.

Certainly some of the basis for these effects of gene deletion might be rationalized by examining the effects of ethanol on dopamine neurons. Ethanol stimulates firing of dopaminergic neurons (Mereu *et al*, 1984; Mereu and Gessa, 1984; Brodie *et al*, 1990, 1999) and increases extracellular levels of DA in the nucleus accumbens (Wozniak *et al*, 1991; Yoshimoto *et al*, 1992a, b; Kiianmaa *et al*, 1995). Ethanol supports self-administration behavior when injected directly into the ventral tegmental area, consistent with a large role of dopaminergic systems in ethanol reward (Gatto *et al*, 1994). However, in considering the potential consequences of DAT and VMAT2 gene KO on ethanol actions, the effects of these gene KOs on monoamine neurotransmitter dynamics should also be considered.

DAT KO mice display not only DAT loss but also compensatory reductions in dopamine synthesis (Jaber *et al*, 1999), autoreceptor function (Jones *et al*, 1999), and dopamine receptor levels (Sora *et al*, 2001b), making simple predictions difficult. This circumstance is even more complicated in heterozygous KOs in which compensatory potential is greater than in full KOs, depending on the degree of compensatory change and receptor reserve (Sora *et al*, 2001a). Such complications could account for examples of enhanced pharmacological effects in heterozygous KO mice (eg Figure 4b; Sora *et al*, 2001a). In any case, the simplest hypothesis is that reduced DA reuptake could potentiate the effects of ethanol-induced firing of VTA neurons by prolonging the time during which released dopamine is available to interact with extracellular receptors and by extending the extracellular distance that released dopamine can travel before it is inactivated by uptake or metabolism.

Since VMAT2 KO reduces the accumulation of all monoamines into vesicles, multiple effects of VMAT2 KO could affect ethanol consumption. VMAT2 KOs alter monoamine function by reducing tissue content (Fon *et al*, 1997; Wang *et al*, 1997; Mooslehner *et al*, 2001), extracellular monoamine levels (Wang *et al*, 1997), and the amounts of monoamine that amphetamine or depolar-

ization can release (Wang *et al*, 1997). VMAT2 +/− mice display enhanced locomotor effects of psychostimulants (Takahashi and Uhl, 1997) and ethanol (Wang *et al*, 1997), but reduced reward from amphetamine (Takahashi *et al*, 1997). While DAT and VMAT2 reductions can produce dissimilar effects, both may be seen as reducing the influence of 'phasic' levels of dopamine released by nerve impulse trains in relationship to the relatively increased influence of 'tonic' dopamine whose levels and distributions are less dependent on dopamine cell firing. While these considerations provide a plausible shared dopamine mechanism for the effects of DAT and VMAT2 KO, neither the effects of VMAT2 KO or DAT KO on ethanol consumption are necessarily or entirely mediated by direct alterations in dopaminergic function.

The effects of DAT and VMAT2 KO on ethanol consumption were sex dependent. Female mice have often been observed to consume more ethanol than male mice (Middaugh and Kelley, 1999; Middaugh *et al*, 1999), and the effects of MOR gene KO are also sex dependent (Hall *et al*, 2001). As modulation of MOR levels by progesterone and estrogen (Carter and Soliman, 1996, 1998) provides one potential explanation for the sex interaction with MOR KO, so ovarian hormonal regulation of DAT and VMAT2 (Attali *et al*, 1997; Disshon *et al*, 1998) might also account for sex-dependent effects in DAT and VMAT2 KO mice. In addition, different consumption levels in male and female mice might affect the sensitivity with which these experiments could reveal enhanced or reduced ethanol consumption. Higher consumption may reduce the likelihood of detecting elevated ethanol consumption (ie ceiling effects), as noted for the female mice in this study. This analysis also suggests that other factors that increase or decrease ethanol consumption, such as the background strain of the mice (eg C57Bl/6 *vs* DBA/2), might similarly interact with the effects of gene KO.

In addition to the dependency on sex, the effects of genotype were also highly dependent on ethanol concentration. The effects of genotype were only observed at high ethanol concentrations. Such concentration dependencies have been noted previously in the effects of isolation rearing (Wolffgramm, 1990; Hall *et al*, 1998) and strain (Hall *et al*, 1998) on ethanol consumption in rats. The reasons for concentration dependencies are unknown; however it might be speculated that if ethanol produces rewarding effects through multiple mechanisms, then this might be differentially activated by different doses of ethanol. Alternatively, this might be due to differences in taste aversion, although such suggestions have not proved true for the strain differences mentioned earlier (Hall *et al*, 1998).

Observations in heterozygous mice, in which the expression of VMAT2 and DAT lie within the range of human variation in the expression of these genes, may have direct relevance to alcoholism, although substantial environmental impact on human individual differences in the expression of these genes is still possible. A human DAT exon 15 variable number tandem repeat marker (VNTR) has been frequently examined in association studies that have compared alcoholics or polysubstance abusers with controls. This VNTR DAT polymorphism has been associated with modestly altered DAT availability as assessed by single photon emission computed tomography (SPECT) in alcoholics and controls (Heinz *et al*, 2000). Although several significant associations (Muramatsu and Higuchi, 1995; Dobashi *et al*, 1997; Sander *et al*, 1997b; Ueno *et al*, 1999) have been reported, most studies lack such association (Parsian and Zhang, 1997; Franke *et al*, 1999; Heinz *et al*, 2000), although no study carefully examined possible sex effects. Furthermore, the VNTR marker displays little linkage disequilibrium with 5′ exons or 5′ flanking sequences that are more classical candidates to control levels of DAT expression (Vandenbergh *et al*, 2000). Initial human VMAT2 variants have also been studied for association with polysubstance abuse vulnerability, but these markers again are unlikely to adequately reflect all of the important variation identified at this locus (Uhl *et al*, 2000). The current observations of sex-dependent differences in ethanol consumption or preference in DAT and VMAT KO mice add to the weight of evidence for monoaminergic influences on alcohol consumption and motivate more careful evaluation of sex-dependent differences at these loci in humans of genetic vulnerability to alcoholism.

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REFERENCES

Arora RC, Tong C, Jackman HL, Stoff D, Meltzer HY (1983). Serotonin uptake and imipramine binding in blood platelets and brain of Fawn-Hooded and Sprague–Dawley rats. *Life Sci* **33**: 437–442.

Attali G, Weizman A, Gil-Ad I, Rehavi M (1997). Opposite modulatory effects of ovarian hormones on rat brain dopamine and serotonin transporters. *Brain Res* **756**: 153–159.

Aulakh CS, Hill JL, Lesch KP, Murphy DL (1992). Functional subsensitivity of 5-hydroxytryptamine1C or alpha 2 adrenergic heteroreceptors mediating clonidine-induced growth hormone release in the Fawn-Hooded rat strain relative to the Wistar rat strain. *J Pharmacol Exp Ther* **262**: 1038–1043.

Aulakh CS, Hill JL, Murphy D (1988a). A comparison of feeding and locomotion responses to serotonin agonists in three rat strains. *Pharmacol Biochem Behav* **31**: 567–571.

Aulakh CS, Tolliver T, Wozniak KM, Hill JL, Murphy DL (1994). Functional and biochemical evidence for altered serotonergic function in the Fawn-Hooded rat strain. *Pharmacol Biochem Behav* **49**: 615–620.

Aulakh CS, Wozniak KM, Hill JL, Devane CL, Tolliver TJ, Murphy DL (1988b). Differential neuroendocrine responses to the 5-HT agonist *m*-chlorophenylpiperazine in Fawn-Hooded rats relative to Wistar and Sprague–Dawley rats. *Neuroendocrinology* **48**: 401–406.

Blomqvist O, Gelernter J, Kranzler HR (2000). Family-based study of DRD2 alleles in alcohol and drug dependence. *Am J Med Genet* **96**: 659–664.

Brodie MS, Pesold C, Appel SB (1999). Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol Clin Exp Res* **23**: 1848–1852.

Brodie MS, Shefner SA, Dunwiddie TV (1990). Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area *in vitro*. *Brain Res* **508**: 65–69.

Carter A, Soliman MR (1996). Estradiol alters ethanol-induced effects on beta-endorphin and met-enkephalin levels in specific brain regions of ovariectomized rats. *Pharmacology* **53**: 143–150.

Carter A, Soliman MR (1998). Estradiol and progesterone alter ethanol-induced effects on mu-opioid receptors in specific brain regions of ovariectomized rats. *Life Sci* **62**: 93–101.

Chen F, Lawrence AJ (2000). 5-HT transporter sites and 5-HT1A and 5-HT3 receptors in Fawn-Hooded rats: a quantitative autoradiography study. *Alcohol Clin Exp Res* **24**: 1093–1102.

Cloninger CR (1987). Neurogenetic adaptive mechanisms in alcoholism. *Science* **236**: 410–416.

Crabbe JC, Phillips TJ, Feller DJ, Hen R, Wenger CD, Lessov CN *et al* (1996). Elevated alcohol consumption in null mutant mice lacking 5-HT1B serotonin receptors. *Nat Genet* **14**: 98–101.

Disshon KA, Boja JW, Dluzen DE (1998). Inhibition of striatal dopamine transporter activity by 17beta-estradiol. *Eur J Pharmacol* **345**: 207–211.

Dobashi I, Inada T, Hadano K (1997). Alcoholism and gene polymorphisms related to central dopaminergic transmission in the Japanese population. *Psychiatr Genet* **7**: 87–91.

Donovan DM, Vandenbergh DJ, Perry MP, Bird GS, Ingersoll R, Nanthakumar E *et al* (1995). Human and mouse dopamine transporter genes: conservation of 5′-flanking sequence elements and gene structures. *Brain Res Mol Brain Res* **30**: 327–335.

Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM (1993). Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. *Alcohol* **10**: 207–212.

El-Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T *et al* (1998). Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur J Pharmacol* **353**: 149–158.

Erickson JD, Schafer MK, Bonner TI, Eiden LE, Weihe E (1996). Distinct pharmacological properties and distribution in neurons and endocrine cells of two isoforms of the human vesicular monoamine transporter. *Proc Natl Acad Sci USA* **93**: 5166–5171.

Fon EA, Pothos EN, Sun BC, Killeen N, Sulzer D, Edwards RH (1997). Vesicular transport regulates monoamine storage and release but is not essential for amphetamine action. *Neuron* **19**: 1271–1283.

Franke P, Schwab SG, Knapp M, Gansicke M, Delmo C, Zill P *et al* (1999). DAT1 gene polymorphism in alcoholism: a family-based association study. *Biol Psychiatry* **45**: 652–654.

Gasnier B (2000). The loading of neurotransmitters into synaptic vesicles. *Biochimie* **82**: 327–337.

Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li TK (1994). Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* **11**: 557–564.

Gelernter J, Kranzler H (1999). D2 dopamine receptor gene (DRD2) allele and haplotype frequencies in alcohol dependent and control subjects: no association with phenotype or severity of phenotype. *Neuropsychopharmacology* **20**: 640–649.

Gelernter J, Kranzler H, Cubells JF (1997). Serotonin transporter protein (SLC6A4) allele and haplotype frequencies and linkage disequilibria in African- and European–American and Japanese populations and in alcohol-dependent subjects. *Hum Genet* **101**: 243–246.

Gelernter J, O'Malley S, Risch N, Kranzler HR, Krystal J, Merikangas K (1991). No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. *JAMA* **266**: 1801–1807.

Gianoulakis C (2001). Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. *J Psychiatry Neurosci* **26**: 304–318.

Gilligan SB, Reich T, Cloninger CR (1987). Etiologic heterogeneity in alcoholism. *Genet Epidemiol* **4**: 395–414.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC (1997). Linkage and association of a functional DRD2 variant [Ser311Cys] and DRD2 markers to alcoholism, substance abuse and schizophrenia in Southwestern American Indians. *Am J Med Genet* **74**: 386–394.

Goldman D, Urbanek M, Guenther D, Robin R, Long JC (1998). A functionally deficient DRD2 variant [Ser311Cys] is not linked to alcoholism and substance abuse. *Alcohol* **16**: 47–52.

Grace AA (2000). The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction* **95**(Suppl 2): S119–S128.

Gudelsky GA, Koenig JI, Meltzer HY (1985). Altered responses to serotonergic agents in Fawn-Hooded rats. *Pharmacol Biochem Behav* **22**: 489–492.

Hall FS, Huang S, Fong GW, Pert A, Linnoila M (1998). Effects of isolation-rearing on voluntary consumption of ethanol, sucrose and saccharin solutions in Fawn Hooded and Wistar rats. *Psychopharmacology (Berl)* **139**: 210–216.

Hall FS, Sora I, Uhl GR (2001). Ethanol consumption and reward are decreased in mu-opiate receptor knockout mice. *Psychopharmacology (Berl)* **154**: 43–49.

Han C, McGue MK, Iacono WG (1999). Lifetime tobacco, alcohol and other substance use in adolescent Minnesota twins: univariate and multivariate behavioral genetic analyses. *Addiction* **94**: 981–993.

Heath AC, Bucholz KK, Madden PA, Dinwiddie SH, Slutske WS, Bierut LJ *et al* (1997). Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychol Med* **27**: 1381–1396.

Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG *et al* (2000). Genotype influences *in vivo* dopamine transporter availability in human striatum. *Neuropsychopharmacology* **22**: 133–139.

Higley JD, Suomi SJ, Linnoila M (1996). A nonhuman primate model of type II excessive alcohol consumption? Part 1. Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations and diminished social competence correlate with excessive alcohol consumption. *Alcohol Clin Exp Res* **20**: 629–642.

Higuchi S, Muramatsu T, Murayama M, Hayashida M (1994). Association of structural polymorphism of the dopamine D2 receptor gene and alcoholism. *Biochem Biophys Res Commun* **204**: 1199–1205.

Hodge CW, Samson HH, Chappelle AM (1997). Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcohol Clin Exp Res* **21**: 1083–1091.

Hoffman PL, Yagi T, Tabakoff B, Phillips TJ, Kono H, Messing RO *et al* (2001). Transgenic and gene 'knockout' models in alcohol research. *Alcohol Clin Exp Res* **25**: 60S–66S.

Hulihan-Giblin BA, Park YD, Aulakh CS, Goldman D (1992). Regional analysis of 5-HT1A and 5-HT2 receptors in the Fawn-Hooded rat. *Neuropharmacology* **31**: 1095–1099.

Hulihan-Giblin BA, Park YD, Goldman D, Aulakh CS (1993). Analysis of the 5-HT1C receptor and the serotonin uptake site in Fawn-Hooded rat brain. *Eur J Pharmacol* **239**: 99–102.

Ikemoto S, McBride WJ, Murphy JM, Lumeng L, Li TK (1997). 6-OHDA-lesions of the nucleus accumbens disrupt the acquisition but not the maintenance of ethanol consumption in the alcohol-preferring P line of rats. *Alcohol Clin Exp Res* **21**: 1042–1046.

Iwata N, Virkkunen M, Linnoila M, Goldman D (1998). Identification of a naturally occurring Pro15-Ser15 substitution in the

serotonin5A receptor gene in alcoholics and healthy volunteers. *Brain Res Mol Brain Res* **58**: 217–220.

Jaber M, Dumartin B, Sagne C, Haycock JW, Roubert C, Giros B *et al* (1999). Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter. *Eur J Neurosci* **11**: 3499–3511.

Jones SR, Gainetdinov RR, Hu XT, Cooper DC, Wightman RM, White FJ *et al* (1999). Loss of autoreceptor functions in mice lacking the dopamine transporter. *Nat Neurosci* **2**: 649–655.

Kiianmaa K, Nurmi M, Nykanen I, Sinclair JD (1995). Effect of ethanol on extracellular dopamine in the nucleus accumbens of alcohol-preferring AA and alcohol-avoiding ANA rats. *Pharmacol Biochem Behav* **52**: 29–34.

Laine TP, Ahonen A, Rasanen P, Pohjalainen T, Tiihonen J, Hietala J (2001a). The A1 allele of the D2 dopamine receptor gene is associated with high dopamine transporter density in detoxified alcoholics. *Alcohol Alcohol* **36**: 262–265.

Laine TP, Ahonen A, Rasanen P, Tiihonen J (2001b). Dopamine transporter density and novelty seeking among alcoholics. *J Addict Dis* **20**: 91–96.

Lancaster FE (1994). Gender differences in the brain: implications for the study of human alcoholism. *Alcohol Clin Exp Res* **18**: 740–746.

Lancaster FE (1995). Gender differences in animal studies. Implications for the study of human alcoholism. *Recent Dev Alcohol* **12**: 209–215.

Lappalainen J, Long JC, Eggert M, Ozaki N, Robin RW, Brown GL *et al* (1998). Linkage of antisocial alcoholism to the serotonin 5-HT1B receptor gene in 2 populations. *Arch Gen Psychiatry* **55**: 989–994.

Lappalainen J, Long JC, Virkkunen M, Ozaki N, Goldman D, Linnoila M (1999). HTR2C Cys23Ser polymorphism in relation to CSF monoamine metabolite concentrations and DSM-III-R psychiatric diagnoses. *Biol Psychiatry* **46**: 821–826.

Levy AD, Murphy JM, McBride WJ, Lumeng L, Li TK (1991). Microinjection of sulpiride into the nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats. *Alcohol Alcohol* **1**(Suppl): 417–420.

Li TK (2000). Pharmacogenetics of responses to alcohol and genes that influence alcohol drinking. *J Stud Alcohol* **61**: 5–12.

Linnoila M, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK (1983). Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. *Life Sci* **33**: 2609–2614.

Lovinger DM (1999). 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. *Neurochem Int* **35**: 125–130.

Matsushita S, Muramatsu T, Murayama M, Nakane J, Higuchi S (2001). Alcoholism, ALDH2*2 allele and the A1 allele of the dopamine D2 receptor gene: an association study. *Psychiatry Res* **104**: 19–26.

McBride WJ, Chernet E, Dyr W, Lumeng L, Li TK (1993). Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. *Alcohol* **10**: 387–390.

McBride WJ, Chernet E, Russell RN, Wong DT, Guan XM, Lumeng L *et al* (1997). Regional CNS densities of monoamine receptors in alcohol-naive alcohol-preferring P and nonpreferring NP rats. *Alcohol* **14**: 141–148.

McBride WJ, Guan XM, Chernet E, Lumeng L, Li TK (1994). Regional serotonin1A receptors in the CNS of alcohol-preferring and nonpreferring rats. *Pharmacol Biochem Behav* **49**: 7–12.

McBride WJ, Murphy JM, Gatto GJ, Levy AD, Lumeng L, Li TK (1991). Serotonin and dopamine systems regulating alcohol intake. *Alcohol Alcohol* **1**(Suppl): 411–416.

McBride WJ, Murphy JM, Lumeng L, Li TK (1990). Serotonin, dopamine and GABA involvement in alcohol drinking of selectively bred rats. *Alcohol* **7**: 199–205.

Mereu G, Fadda F, Gessa GL (1984). Ethanol stimulates the firing rate of nigral dopaminergic neurons in unanesthetized rats. *Brain Res* **292**: 63–69.

Mereu G, Gessa GL (1984). Ethanol excites dopamine (DA) neurons and inhibits non-dopamine (non-DA) neurons in the Substantia nigra of rats. *Ann Ist Super Sanita* **20**: 11–15.

Middaugh LD, Kelley BM (1999). Operant ethanol reward in C57BL/6 mice: influence of gender and procedural variables. *Alcohol* **17**: 185–194.

Middaugh LD, Kelley BM, Bandy AL, McGroarty KK (1999). Ethanol consumption by C57BL/6 mice: influence of gender and procedural variables. *Alcohol* **17**: 175–183.

Mooslehner KA, Chan PM, Xu W, Liu L, Smadja C, Humby T *et al* (2001). Mice with very low expression of the vesicular monoamine transporter 2 gene survive into adulthood: potential mouse model for parkinsonism. *Mol Cell Biol* **21**: 5321–5331.

Muramatsu T, Higuchi S (1995). Dopamine transporter gene polymorphism and alcoholism. *Biochem Biophys Res Commun* **211**: 28–32.

Myers RD, Melchior CL (1977). Alcohol and alcoholism: role of serotonin. In: Essman WB (ed). *Physiol Regulation Pharmacol Action*, Vol. 2. Spectrum: New York. pp 373–430.

Myrick H, Brady KT, Malcolm R (2001). New developments in the pharmacotherapy of alcohol dependence. *Am J Addict* **10**: 3–15.

Nielsen DA, Virkkunen M, Lappalainen J, Eggert M, Brown GL, Long JC *et al* (1998). A tryptophan hydroxylase gene marker for suicidality and alcoholism. *Arch Gen Psychiatry* **55**: 593–602.

Nutt D (1999). Alcohol and the brain. Pharmacological insights for psychiatrists. *Br J Psychiatry* **175**: 114–119.

Parsian A, Zhang ZH (1997). Human dopamine transporter gene polymorphism (VNTR) and alcoholism. *Am J Med Genet* **74**: 480–482.

Pesonen U, Koulu M, Bergen A, Eggert M, Naukkarinen H, Virkkunen M *et al* (1998). Mutation screening of the 5-hydroxytryptamine7 receptor gene among Finnish alcoholics and controls. *Psychiatry Res* **77**: 139–145.

Phillips TJ, Brown KJ, Burkhart-Kasch S, Wenger CD, Kelly MA, Rubinstein M *et al* (1998). Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nat Neurosci* **1**: 610–615.

Risinger FO, Bormann NM, Oakes RA (1996). Reduced sensitivity to ethanol reward, but not ethanol aversion, in mice lacking 5-HT1B receptors. *Alcohol Clin Exp Res* **20**: 1401–1405.

Risinger FO, Doan AM, Vickrey AC (1999). Oral operant ethanol self-administration in 5-HT1b knockout mice. *Behav Brain Res* **102**: 211–215.

Risinger FO, Freeman PA, Rubinstein M, Low MJ, Grandy DK (2000). Lack of operant ethanol self-administration in dopamine D2 receptor knockout mice. *Psychopharmacology (Berl)* **152**: 343–350.

Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G *et al* (1997). Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. *Cell* **90**: 991–1001.

Russell RN, McBride WJ, Lumeng L, Li TK, Murphy JM (1996). Apomorphine and 7-OH DPAT reduce ethanol intake of P and HAD rats. *Alcohol* **13**: 515–519.

Sander T, Harms H, Dufeu P, Kuhn S, Hoehe M, Lesch KP *et al* (1998). Serotonin transporter gene variants in alcohol-dependent subjects with dissocial personality disorder. *Biol Psychiatry* **43**: 908–912.

Sander T, Harms H, Dufeu P, Kuhn S, Rommelspacher H, Schmidt LG (1997a). Dopamine D4 receptor exon III alleles and variation of novelty seeking in alcoholics. *Am J Med Genet* **74**: 483–487.

Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A (1997b). Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. *Biol Psychiatry* **41**: 299–304.

Sander T, Syagailo Y, Samochowiec J, Okladnova O, Lesch KP, Janz D (1999). Association analysis of a regulatory promoter polymorphism of the PAX-6 gene with idiopathic generalized epilepsy. *Epilepsy Res* **36**: 61–67.

Savelieva KV, Caudle WM, Findlay GS, Caron MG, Miller GW (2002). Decreased ethanol preference and consumption in dopamine transporter female knock-out mice. *Alcohol Clin Exp Res* **26**: 758–764.

Schmidt LG, Sander T, Kuhn S, Smolka M, Rommelspacher H, Samochowiec J *et al* (2000). Different allele distribution of a regulatory MAOA gene promoter polymorphism in antisocial and anxious-depressive alcoholics. *J Neural Transm* **107**: 681–689.

Schuckit MA, Mazzanti C, Smith TL, Ahmed U, Radel M, Iwata N *et al* (1999). Selective genotyping for the role of 5-HT2A, 5-HT2C, and GABA alpha 6 receptors and the serotonin transporter in the level of response to alcohol: a pilot study. *Biol Psychiatry* **45**: 647–651.

Sora I, Elmer G, Funada M, Pieper J, Li XF, Hall FS *et al* (2001a). Mu opiate receptor gene dose effects on different morphine actions: evidence for differential *in vivo* mu receptor reserve. *Neuropsychopharmacology* **25**: 41–54.

Sora I, Hall FS, Andrews AM, Itokawa M, Li XF, Wei HB *et al* (2001b). Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference. *Proc Natl Acad Sci USA* **98**: 5300–5305.

Sora I, Wichems C, Takahashi N, Li XF, Zeng Z, Revay R *et al* (1998). Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci USA* **95**: 7699–7704.

Stefanini E, Frau M, Garau MG, Garau B, Fadda F, Gessa GL (1992). Alcohol-preferring rats have fewer dopamine D2 receptors in the limbic system. *Alcohol Alcohol* **27**: 127–130.

Takahashi N, Miner LL, Sora I, Ujike H, Revay RS, Kostic V *et al* (1997). VMAT2 knockout mice: heterozygotes display reduced amphetamine-conditioned reward, enhanced amphetamine locomotion, and enhanced MPTP toxicity. *Proc Natl Acad Sci USA* **94**: 9938–9943.

Takahashi N, Uhl G (1997). Murine vesicular monoamine transporter 2: molecular cloning and genomic structure. *Brain Res Mol Brain Res* **49**: 7–14.

Ueno S, Nakamura M, Mikami M, Kondoh K, Ishiguro H, Arinami T *et al* (1999). Identification of a novel polymorphism of the human dopamine transporter (DAT1) gene and the significant association with alcoholism. *Mol Psychiatry* **4**: 552–557.

Uhl GR (1998). Hypothesis: the role of dopaminergic transporters in selective vulnerability of cells in Parkinson's disease. *Ann Neurol* **43**: 555–560.

Uhl GR, Li S, Takahashi N, Itokawa K, Lin Z, Hazama M *et al* (2000). The VMAT2 gene in mice and humans: amphetamine responses, locomotion, Cardiac arrhythmias, aging, and vulnerability to dopaminergic toxins. *FASEB J* **14**: 2459–2465.

Vandenbergh DJ, Thompson MD, Cook EH, Bendahhou E, Nguyen T, Krasowski MD *et al* (2000). Human dopamine transporter gene: coding region conservation among normal, Tourette's disorder, alcohol dependence and attention-deficit hyperactivity disorder populations. *Mol Psychiatry* **5**: 283–292.

Wang P, Aulakh CS, Hill JL, Murphy DL (1988). Fawn-Hooded rats are subsensitive to the food intake suppressant effects of 5-HT agonists. *Psychopharmacology* **94**: 558–562.

Wang YM, Gainetdinov RR, Fumagalli F, Xu F, Jones SR, Bock CB *et al* (1997). Knockout of the vesicular monoamine transporter 2 gene results in neonatal death and supersensitivity to cocaine and amphetamine. *Neuron* **19**: 1285–1296.

Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP *et al* (2001). Compulsive drug-seeking behavior and relapse. Neuroadaptation, stress, and conditioning factors. *Ann NY Acad Sci* **937**: 1–26.

Wolffgramm J (1990). Free choice ethanol intake of laboratory rats under different social conditions. *Psychopharmacology* **101**: 233–239.

Wong DT, Reid LR, Li TK, Lumeng L (1993). Greater abundance of serotonin1A receptor in some brain areas of alcohol-preferring (P) rats compared to nonpreferring (NP) rats. *Pharmacol Biochem Behav* **46**: 173–177.

Wozniak KM, Pert A, Linnoila M (1990). Antagonism of 5-HT3 receptors attenuates the effects of ethanol on extracellular dopamine. *Eur J Pharmacol* **187**: 287–289.

Wozniak KM, Pert A, Mele A, Linnoila M (1991). Focal application of alcohols elevates extracellular dopamine in rat brain: a microdialysis study. *Brain Res* **540**: 31–40.

Yan QS, Reith ME, Jobe PC, Dailey JW (1996). Focal ethanol elevates extracellular dopamine and serotonin concentrations in the rat ventral tegmental area. *Eur J Pharmacol* **301**: 49–57.

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1992a). Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. *Alcohol* **9**: 17–22.

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1992b). Ethanol enhances the release of dopamine and serotonin in the nucleus accumbens of HAD and LAD lines of rats. *Alcohol Clin Exp Res* **16**: 781–785.

Zhou FC, Pu CF, Murphy J, Lumeng L, Li TK (1994). Serotonergic neurons in the alcohol preferring rats. *Alcohol* **11**: 397–403.

Zhou FC, Zhang JK, Lumeng L, Li TK (1995). Mesolimbic dopamine system in alcohol-preferring rats. *Alcohol* **12**: 403–412.