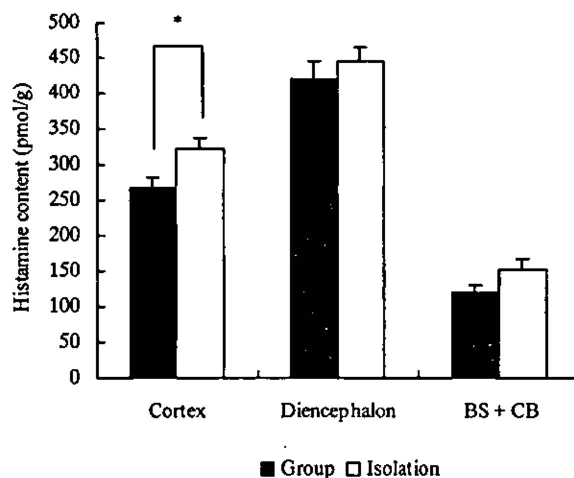

FIGURE 4. Histamine content in the brain of METH-treated mice. The histamine content was compared between socially isolated and grouped mice. Data are expressed as the mean ± SEM of 10 mice in each group. Statistical significance of difference between the two groups was analyzed by the Mann-Whitney test. $^*P < .05$.

Chronic Stressful Conditions and the Histaminergic Neuron System

Previous animal studies reported that neuronal histamine is released in the acute phase of stress.[9–11] We previously reported the chronological changes of the central histaminergic system of rats subjected to chronic stress induced by food deprivation and physical activity on a running wheel.[12] The H3 receptor density rapidly declined in the acute phase of food-deprived activity stress, but gradually returned to the control level in the chronic phase. The initial decrease in H3 receptor bindings would simply reflect the increased histamine release by acute stress. In the late stage, the density of H1 receptors decreased significantly while the decreased levels of H3 receptors returned to normal. In the late stages, the release of histamine from neurons by stress reaction gradually decreased in addition to the increase of histamine content. These changes presumably resulted in the inhibition of the net histaminergic neuronal activity in the chronic stress condition. Similar changes in histaminergic neurotransmission could be generated in social isolation stress. In conclusion, the present study demonstrated that postnatal social isolation accelerated the effects of repeated injections of METH on behavioral sensitization in parallel with neurochemical changes. The central histaminergic system might be deactivated during chronic stressful conditions in the same way as are the dopaminergic and serotonergic systems.

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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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Keywords: transgenic mice; dopamine transporter; vesicular monoamine transporter; ethanol; reward

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.

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, $p < 0.05$.

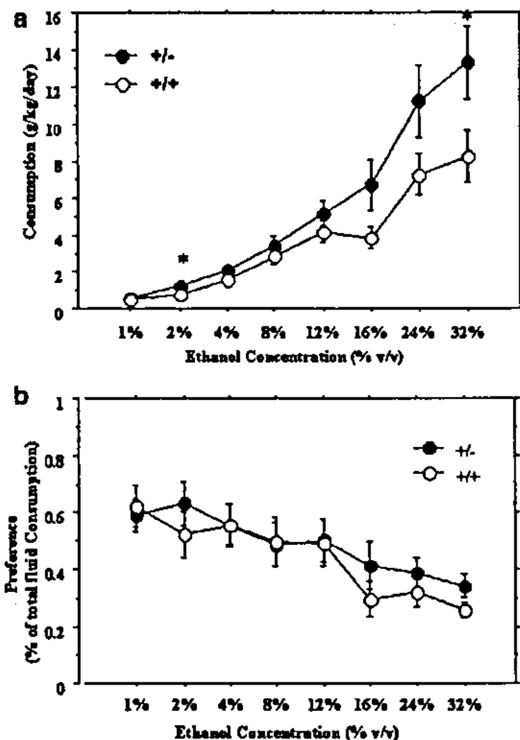

Figure 1 Male VMAT2 KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for male +/+, and +/− VMAT2 KO mice. The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post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

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 $F[1,56] = 6.8$, $p < 0.02$).

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

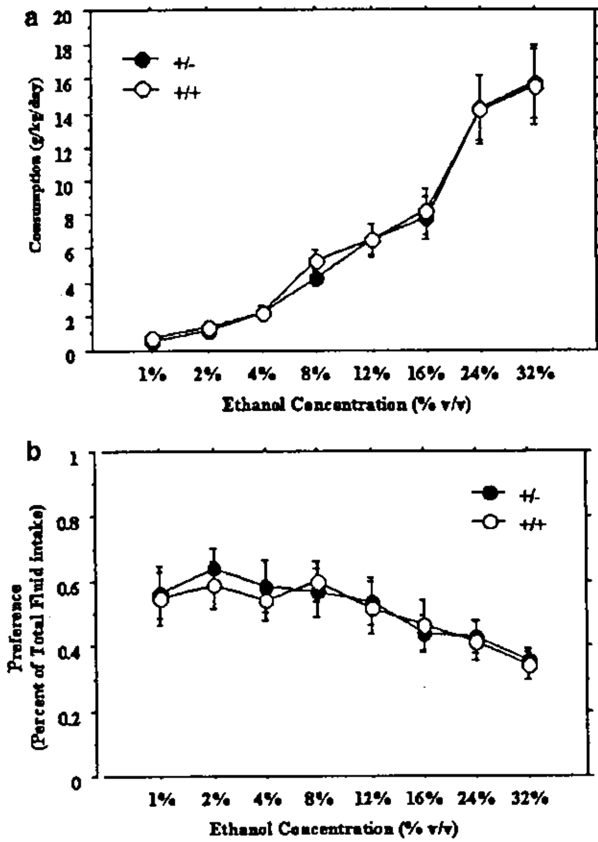

Figure 2 Female VMAT2 KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for female +/+, and +/− VMAT2 KO mice. The data are expressed as mean ± SEM.

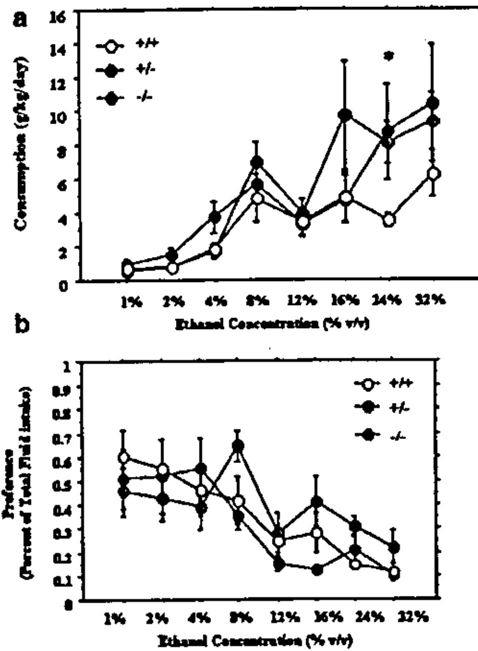

Figure 3 Male DAT KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for male +/+, +/−, and −/− DAT KO mice. The data are expressed as mean ± SEM. *Significant *post hoc* difference using Scheffe's *post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

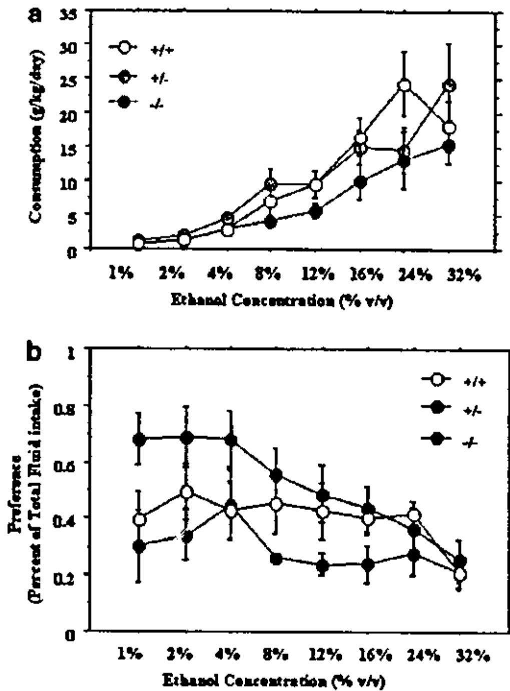

Figure 4 Female DAT KO mice: The data represent voluntary consumption of ethanol in grams per kilogram of body weight per day (g/kg/day) (a) and percent preference (b), for female +/+, +/−, and −/− DAT KO mice. The data are expressed as mean ± SEM.

($F[2,45]=4.0$, $p<0.03$). 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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μ-Opioid receptor knockout mice display reduced cocaine conditioned place preference but enhanced sensitization of cocaine-induced locomotion

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Abstract

The μ-opioid receptor (OPRM1) is expressed in brain regions implicated in reward and locomotor processes. Reduced reward, not only from opiates, but also from several other abused substances has been observed in mice with lifelong deletions of the OPRM1 gene. To further define the roles of μ-opioid receptors in psychostimulant actions, cocaine psychomotor stimulant and rewarding effects were examined in wild-type (WT), heterozygous and homozygous μ-opioid receptor knockout mice. While μ-opioid receptor knockout did not affect basal locomotion, locomotor stimulant effects of cocaine were enhanced in a within-subjects dose–response experiment. However, further study revealed that in mice injected with 20 mg/kg for the first time, there was no difference in the locomotor-stimulating effects of cocaine between knockout and wild-type mice. In a sensitization study (modeled after the conditions in the dose–response experiment) although not observed in WT mice, OPRM1 −/− mice did exhibit cocaine sensitization. By stark contrast, and similar to the effects of other rewarding drugs in OPRM1 KO mice, cocaine reward, as assessed by conditioned place preference, was reduced in both homozygous and heterozygous OPRM1 KO mice. The present results confirm a central role of the μ-opioid receptor in drug reward but opposing effects on locomotor sensitization. The reduced cocaine reward identified in heterozygous μ-opioid receptor knockout mice supports the possibility that humans with fewer available μ-opioid receptors might experience less cocaine reward.
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1. Introduction

Cocaine is a psychostimulant that inhibits the reuptake of dopamine, serotonin and norepinephrine by blocking their respective transporters, DAT, SERT and NET. Roles for cocaine actions at several transporters in reward mechanisms are supported by recent observations that combined knockouts of DAT and SERT eliminate cocaine conditioned place preference (CPP), while knockout of DAT alone, SERT alone, NET alone, or combined knockout of SERT and NET are ineffective or actually enhance cocaine CPP [23,64,66,77].

Circuits important for cocaine reward and locomotion are not likely to be limited to those using monoaminergic neurotransmitters, however (see [25] for review). Neurons that express preproenkephalin peptides and μ-opioid receptors are present in much of the brain's "reward" and locomotor circuitry, including afferents to the ventral tegmental area and neuronal cell bodies in the nucleus accumbens and caudate/putamen [39,50,68,73,74]. Many authors have reviewed evidence supporting the possibility that these opioidergic systems play roles in the reward elicited by administration of psychomotor stimulants and other abused substances (for review, see Refs. [26,33,41,42,69]). Neurons that express preprodynorphin peptides and kappa opioid receptors are also located in many of these same circuits, although these are often localized in cells that are positioned

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to antagonize rewarding or locomotor effects of preproenkephalin/μ-opioid receptor signaling.

Studies with μ-opioid receptor antagonists and partial agonists implicate roles for these opioidergic circuits in psychostimulant reward [41]. The opioid antagonist naloxone can attenuate the acquisition and expression of cocaine conditioned place preference [20] and reduce cocaine self-administration [34]. Although this agonist is not completely selective for the μ-opioid receptor, its aversive effects appear to be entirely μ receptor-mediated [61]. Similar effects are also seen with the selective μ antagonist naloxonazine [55]. The role of the μ-opioid receptor in cocaine-mediated behaviors may be more complex however, as blockade of many of its effects are more consistently observed with the mixed agonist/antagonist buprenorphine [6,9,10,32,35,38,43–46,60,76], which also seems to have greater anti-cocaine potency in at least some clinical studies [47].

We and others have developed OPRM1 knockout mice that consistently display reduced rewarding and locomotor stimulant effects of μ agonists [1,40,66]. Reward and/or self-administration of other addictive substances is also reduced or eliminated by OPRM1 knockout, as assessed by self-administration and/or conditioned place preference procedures: using ethanol [2,22,57], Δ9-tetrahydrocannabinol [21] and cocaine [2]. The locomotor stimulant effects of an acute cocaine injection were reported to be reduced in OPRM1 knockout mice compared to wild-type controls in one study [78], but unaltered in another [2]. While Yoo et al. [78] demonstrated fewer effects of acute cocaine administration to OPRM1 knockout mice, they identified accelerated sensitization of cocaine-induced locomotion.

We now report assessment of the acute locomotor stimulant effects of cocaine locomotion, sensitization of this locomotor response with repeated administration, and cocaine conditioned place preference in wild-type, heterozygous and homozygous OPRM1 knockout mice that we have developed and extensively characterized [18,24,27,29,37,48,49,51,54,62,63,65,66]. Effects in these knockout mice provide an indication of the surprisingly large, and differential, involvement of these opioidergic systems in cocaine reward and locomotor sensitization. We discuss observations in heterozygous mice that express half of the wild-type complement of μ-opioid receptors in light of the range of human variation in these sites.

2. Methods

2.1. Animals

The OPRM1 KO strain used in this report, in which the first exon of the OPRM1 gene has been deleted, has been described previously [63]. OPRM1 KO mice were generated by +/− × +/− crosses of mice from this strain (>10th generation) producing +/+, +/− and −/− littermates. Mice were genotyped by PCR of tail biopsy tissue. The mice were housed at 24 °C with a 12:12-h light/dark cycle and ad libitum access to food and water. All experiments were conducted on mice between 12 and 20 weeks of age. Both male and female mice were used but sex was not evaluated as a factor in any analyses. There was no obvious evidence for any large sex-dependent differences on these measures. Separate groups of mice were tested for the behavioral effects of cocaine in the conditioned place preference paradigm (N=13–16 mice/genotype/dose), baseline locomotion (N=9–11 mice/genotype), cocaine-stimulated locomotion in a within-subjects dose–response design (N=18–21 mice/genotype), cocaine-stimulated locomotion in a between subjects design (N=11–16 mice/condition), and sensitization of the locomotor stimulant effects of cocaine (N=7–8 mice/condition). All experiments were conducted during the light phase of the cycle, between 9 AM and 3 PM.

2.2. Conditioned place preference

Reward was assessed by conditioned place preference testing using a two compartment Plexiglas chamber [66]. Briefly, one compartment (18 × 18 × 18 cm) had a wire mesh floor (1.3 cm grids) mounted over Plexiglas, and the other compartment (18 × 18 × 18 cm) had corncob bedding on a smooth Plexiglas floor. A removable Plexiglas wall separated the two sides. For pre- and post-conditioning test sessions, a 5-cm opening in the center wall allowed access to both compartments. During the conditioning sessions, the opening was eliminated to restrict animals to a single compartment. Locomotion and time spent in each compartment was recorded using an Optovarimax animal activity monitoring apparatus (Columbus Instruments, Columbus, OH). Conditioned place preferences were assessed by determination of compartment preference in three phases. Initial preference was determined as the side in which a mouse spent more than 600 s out of a 20-min trial. Conditioning was conducted over a 2-day period in which cocaine hydrochloride (0, 5 or 10 mg/kg, s.c.) was administered to the animal when it was confined for 30 min in the initially non-preferred compartment and saline administered to the animal when restricted for 30 min to the initially preferred compartment. Animals received two conditioning sessions per day, counterbalanced between saline and cocaine for a total of two saline pairings and two cocaine pairings (or four saline pairings). Conditioned place preference assessment followed the last conditioning session and the last injection by 24 h. Time spent in the drug-paired compartment was compared to the pre-conditioning values obtained on initial assessments.

2.3. Locomotor testing and basal locomotion

Locomotor activity was assessed as total distance traveled. Distance was calculated from measurement of infrared

beam breaks by mice placed individually in 46 × 25 × 19 cm clear plastic cages in Optovarimax activity monitors (Columbus Instruments) under dim light, sound-attenuated conditions. An initial 2-h activity test was conducted in +/+, +/− and −/− OPRM1 mice under novel conditions.

2.4. Cocaine-stimulated locomotion in a within-subjects dose–response design

A separate set of mice were tested for cocaine-stimulated activity using a within-subjects design. After habituation to the apparatus for 1 h mice were injected with cocaine hydrochloride (0, 5, 10 or 20 mg/kg, s.c.), and distance traveled was monitored for an additional 2 h. Doses were counterbalanced across testing days, and at least 48 h separated each testing day. This paradigm, with substantial pre-drug habituation, is commonly used to examine the dose–response characteristics of stimulant drugs because it does not normally produce locomotor sensitization. However, with this possibility in mind two further experiments were conducted.

2.5. Cocaine-stimulated locomotion in a between-subjects design

A separate set of mice was tested for cocaine-stimulated activity using a between-subjects design. Because cocaine did not produce substantial locomotor stimulation in WT mice of this strain, and differences were only observed at the highest doses in the previous experiment (see below), only the effects of cocaine and saline were examined. In each case, each subject was tested only once. After habituation to the apparatus for 2 h mice were injected with either cocaine hydrochloride (20 mg/kg, s.c.) or saline, and the distance traveled was monitored for an additional 2 h.

2.6. Sensitization of locomotor activity

Within-subjects dose–effect curves for locomotor activity are open to the possible confounding effects of locomotor sensitization. Although the within-subjects design utilized in this study minimizes such effects through long habituation prior to drug administration, differences between genotypes observed in within-subjects locomotor testing might possibly reflect differences in locomotor sensitization rather than naive sensitivity to the locomotor-stimulating effects of cocaine. To assess this possibility, separate groups of mice were assessed for sensitization of locomotor activity using a paradigm that closely parallels that used in the dose–response study. Mice were placed in the locomotor testing apparatus as described above each day for 5 days. After 1 h of habituation, mice were injected with either cocaine (20 mg/kg s.c.; this dose was chosen because it was thought that it would produce the maximal amount of sensitization under these conditions) or saline (1 ml/kg) and monitored for 1 h, so that mice were injected five times with cocaine or five times with saline and locomotor responses were assessed.

2.7. Drug

Cocaine HCl (NIDA IRP Drug Supply Program) was dissolved in saline and administered s.c. in a volume of 1.0 ml/kg.

2.8. Statistical analyses

Conditioned place preference was calculated as the difference in time spent on the non-preferred side post-conditioning and the time spent on the non-preferred side pre-conditioning. These data were then subjected to ANOVA with the between subjects factors of GENOTYPE and DOSE.

Baseline locomotor activity data were analyzed by ANOVA with the between subjects factor of GENOTYPE and the within-subjects factor of TIME, with the data divided into 10-min bins. For cocaine-stimulated locomotion the distance traveled was summed over each test session and analyzed by ANOVA with the between subjects factor of GENOTYPE and the within-subjects factor of DRUG (cocaine versus saline). The total locomotor activity after cocaine or saline administration were summed for analysis of sensitization data using the between subjects factors of GENOTYPE and DRUG (cocaine versus saline) and the within-subjects factor of DAY.

Post hoc comparisons were done using Scheffe's test for comparison of two means or by post hoc one-way ANOVA for comparison of dose effects for each GENOTYPE.

3. Results

3.1. Cocaine conditioned place preference

Both doses of cocaine increased preference for the initially non-preferred compartment (Fig. 1; DRUG: $F[2,127]=17.5$, $p<0.0001$). Preference for the compartment associated with either dose of cocaine was reduced to about half of wild-type values in both in OPRM1+/− and OPRM1 −/− knockout mice at both doses of cocaine (Fig. 1; GENOTYPE: $F[2,127]=6.8$, $p<0.01$). The effect of cocaine in OPRM1 −/− mice at the 20 mg/kg dose was not significantly different from saline, and these mice had a significantly reduced preference for the cocaine paired side compared to wild-type littermates ($p<0.05$, Scheffe's comparison).

3.2. Baseline locomotion

All mice exhibited normal elevation of locomotor activity in a novel environment and subsequent habituation of locomotor activity over the period of testing. However,

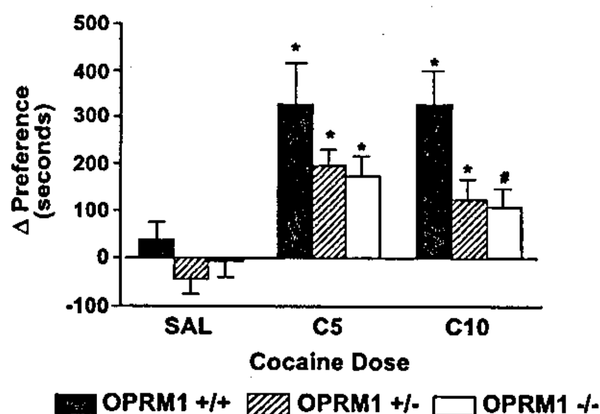

Fig. 1. The conditioned place preference for cocaine is reduced in OPRM1 KO mice. Conditioned place preference induced by cocaine (5 or 10 mg/kg, s.c.; C5 and C10) in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the difference between the pre-conditioning preference and the post-conditioning preference. Data expressed as mean ± standard error of the mean. *Significant difference from saline treatment ($p<0.05$, Scheffe's post hoc comparison). #Significant difference from wild-type mice ($p<0.05$, Scheffe's post hoc comparison).

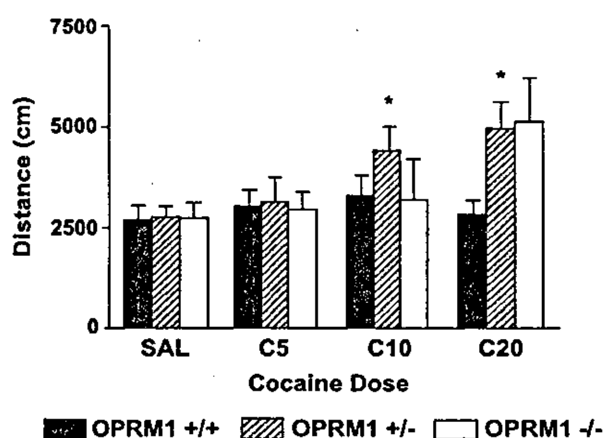

Fig. 3. Within-subjects dose–response study of cocaine-induced locomotor activity in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the total distance traveled over 2 h of testing after the injection (0, 5, 10 and 20 mg/kg cocaine HCl s.c.: SAL, C5, C10 and C20, respectively). Data expressed as mean ± standard error of the mean. *Significant difference from saline based on Scheffe's post hoc comparison ($p<0.05$).

baseline locomotion, tested over a period of 2 h, was not affected by GENOTYPE (Fig. 2; $F[2,29]=0.1$, ns).

3.3. Cocaine-stimulated locomotion in a within-subjects dose–response design

Locomotion during habituation periods prior to drug treatments was not affected by GENOTYPE (data not shown; $F[2,58]=0.1$, ns). Cocaine produced modest but significant locomotor increases that were dependent on DOSE (Fig. 3; $F[2,58]=4.5$, $p<0.01$). Wild-type mice of this strain, of mixed background from the C57/B6 and 129SV progenitor strains, displayed no significant effect of cocaine DOSE (post hoc ANOVA on OPRM1+/+ mice alone: $F[3,62]=0.7$, ns), while a significant effect of cocaine DOSE was observed for OPRM1+/− mice (post hoc ANOVA on OPRM1+/− mice alone: $F[3,51]=3.5$, $p<0.03$) and a trend toward significance was noted in −/− mice (post hoc ANOVA on OPRM1 −/− mice alone: $F[3,60]=2.4$, $p<0.08$).

3.4. Cocaine-stimulated locomotion in a between-subjects design

The locomotor response to cocaine was more robust in this experiment, producing a significant effect of DRUG in

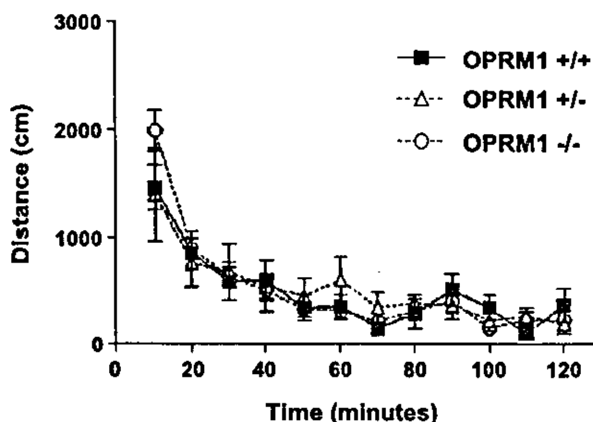

Fig. 2. Time-course of locomotor habituation to a novel environment in OPRM1 KO mice. Time-course of locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed in terms of total distance traveled over the 2-h session. Data expressed as mean ± standard error of the mean.

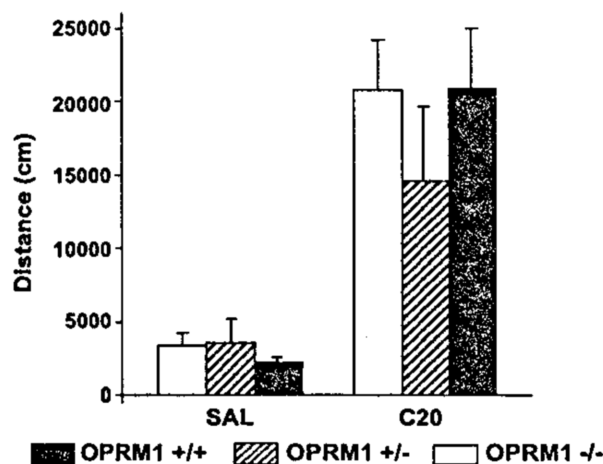

Fig. 4. Between-subjects study of cocaine-induced locomotor activity in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM1+/+, OPRM1+/− and OPRM1 −/− mice expressed as the total distance traveled over 2 h of testing after the injection (0 and 20 mg/kg cocaine HCl s.c.: SAL, C20, respectively). Data expressed as mean ± standard error of the mean.

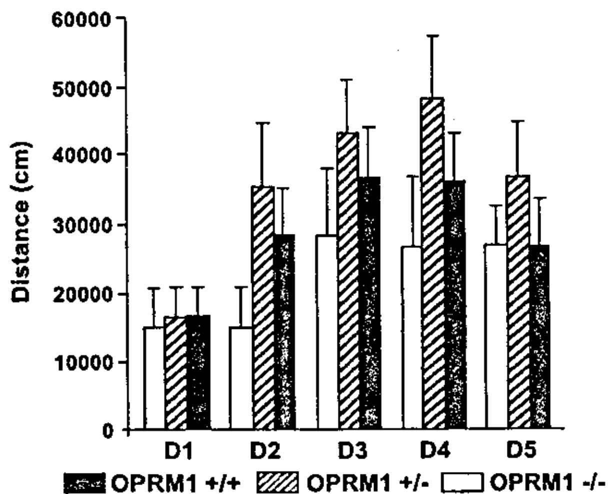

Fig. 5. Sensitization of cocaine-stimulated locomotion in OPRM1 KO mice. Cocaine-induced locomotor activity in OPRM +/+, OPRM +/− and OPRM −/− mice expressed as the total distance travelled over 2 h of testing after cocaine injection (20 mg/kg cocaine HCl s.c.) on five consecutive days (D1–D5). Data expressed as mean ± standard error of the mean.

all groups (Fig. 4; $F[1,73]=35.4, p<0.0001$) but there was no effect of GENOTYPE ($F[2,73]=0.4$, ns).

3.5. Sensitization of cocaine-stimulated locomotion

Under the conditions used in this experiment, cocaine produced substantial locomotor activation (Fig. 5; DRUG, $F[1,45]=51.2$, $p<0.0001$) and there a was significant increase in this locomotor response to cocaine with repeated cocaine administration (DAYS, $F[4,164]=8.3$, $p<0.0001$). However, there was no significant effect of GENOTYPE or a GENOTYPE × DAYS interaction as determined by ANOVA. As in the prior experiment, but in contrast to the within-subjects experiment, there was no effect of GENOTYPE at this dose of cocaine (20 mg/kg) on the first day of administration. Although the ANOVA did not reveal any significant differences, individual post hoc ANOVA of each genotype to determine which genotypes contributed to the significant overall effect of DAYS found significant effects in OPRM1+/− (DAYS, $F[4,56]=4.6$, $p<0.003$) and OPRM1 −/− (DAYS, $F[4,56]=4.2$, $p<0.006$) mice, but not OPRM+/+ mice (DAYS, $F[4,164]=1.6$, ns).

4. Discussion

Lifelong deletion of all or half of the brain's normal complement of μ-opioid receptors reduces the rewarding effects of cocaine in a conditioned place preference paradigm. Findings in heterozygote mice fit well with previously reported data from experiments using doses of opioid antagonists likely to block many, but not all, μ receptors. We discuss these findings in comparison to findings with other drugs, contrasting effects on locomotion and sensitization of locomotion, caveats, implications of the findings for interactions between monoaminergic and opioidergic circuitries controlling reward and locomotor function, and the relevance of these observations for possible effects of human individual differences in μ-opioid receptor expression on psychostimulant reward and locomotion.

The magnitude of the effects of μ-opioid receptor reduction or deletion on reward forms a coherent picture of the role of these receptors in the rewarding effects of multiple classes of abused substances. Deletion of μ-opioid receptors eliminates the conditioned place preference produced by opiates such as morphine, while heterozygous μ opiate receptor knockouts display reduced but significant morphine place preferences [40,66]. These findings have been taken as confirmation that most or all of the rewarding effects of morphine administration depend on its agonist actions at μ-opioid receptors. Most of the place preference for the active cannabinoid, THC, is also eliminated in OPRM1 knockout mice [21]. There are more modest but still significant effects of OPRM1 knockout on conditioned place preference for ethanol [22] and cocaine (Ref. [2] and the present results). Circuits bearing μ-opioid receptors thus seem likely to be crucial for morphine's rewarding actions, highly important for the reward from marijuana's active ingredient, and of significant importance for alcohol and psychostimulant reward. Taken together, these lines of experimental evidence support the idea that brain circuits containing μ-opioid receptors, which may use preproenkephalin peptides as their major endogenous agonists, play a central role in drug-induced reward processes. These data contrast with those from CB1 cannabinoid receptor gene knockouts. These knockouts eliminate the rewarding effects of cannabinoids [36], opiates [13,14,36] (but see Ref. [56]), and nicotine [7] (but see Ref. [13]), but leave the rewarding effects of psychostimulants intact [13,14].

Such a central role for μ-opioid receptors in drug reward does not necessarily mean that this role is limited to the μ-opioid receptors expressed in only a single cell group. Reward can be elicited by opiates microinjected into a number of brain regions [3,4,8,15,16,52]. Reward from systemically administered opiates can be blocked by local injection of opiate antagonists into a number of brain regions [5,11,12,71,72]. Based on this evidence μ-opioid receptors located on afferents to the ventral tegmental area, cells of the nucleus accumbens and caudate/putamen, and even cells and afferents to amygdaloid subnuclei are candidates for roles in μ-opioid receptor-dependent circuits important for reward from each of these substances [39,50,68,73,74]. Several of these nuclei could also be sites for dopamine–opioid interactions. Electron microscopic immunohistochemistry has shown that some single striatal neurons receive both dopaminergic and μ-opioidergic inputs [75], for example. Interaction between dopaminergic and μ-opioidergic neurons in the ventral tegmental area (VTA) allows μ-opioid receptor-mediated

inhibition of inhibitory GABAergic inputs onto VTA dopaminergic neurons [31] and resultant dopaminergic disinhibition. In both of these cases, modulation of dopaminergic tone by peptides interacting with μ-opioid receptors could well modulate the rewarding influences of blockade of dopamine and other monoamine transporters.

Reduced cocaine reward in OPRM1 knockout mice contrasts strongly with increased locomotor activation after the within-subjects dose–response experiments, which appeared to result from increased sensitization to the locomotor stimulant effects of cocaine. Since OPRM1 knockout did not influence the locomotion elicited by a single high cocaine dose in naïve mice, and since the knockouts did influence the results of the sensitization experiment, the increased locomotor activity observed in within-subjects experiments seems likely to be due to genotype-dependent differences in locomotor sensitization. These increased cocaine-sensitized locomotor responses stand in contrast to the reduced rewarding effects of cocaine in the conditioned place preference paradigm. This increased sensitization also contrasts with literature that equates locomotor sensitization with reward system sensitization described in terms of "craving", relapse of drug-seeking and increased incentive motivation [28,58]. Nonetheless, the present data are consistent with the idea that μ-opioid receptor-dependent processes interact with monoaminergic systems differently in producing reward and locomotor sensitization. We have previously identified striking μ-opioid receptor dependence of morphine-stimulated locomotion and reward using the present mouse strains [66]. The current observations also add to prior data from other knockout and transgenic mice that reveal dissociations between acute psychostimulant effects, conditioned place preference, and locomotor sensitization (Refs. [59,64,67]; Randall, Hall and Uhl, unpublished findings, and see Ref. [70] for an overall discussion).

The mechanisms that might underlie such dissociations between different behavioral actions of cocaine in these knockout mice are unknown, but might very well involve effects on other neurotransmitter systems from either chronic changes in opioidergic function or developmental consequences of such changes. Heterozygous OPRM1 knockout mice display 50% of wild-type μ-opioid receptor expression levels, while homozygous OPRM1 knockout mice have no detectable expression [63]. No changes in other opioid receptors are observed [30]. With regard to potential changes in other systems, initial microarray studies suggest that about 1% of the genes studied changed expression more than twofold in the brains of OPRM1 knockout mice (Liu, Uhl and Hall, unpublished findings). Initial observations also suggested that compensatory changes in dopaminergic systems were not prominent in OPRM1 knockout mice [63], although regional analyses have indicated increases in both dopamine D1 receptor and dopamine D2 receptor mRNAs [53].

These data may have implications for the role of μ-opioid receptors in influencing the sensitivity to different effects of cocaine in humans. Several studies of human individual differences in μ-opioid receptor expression in humans suggest the possibility that trait and/or state differences can produce common ranges of individual differences in expression up to the 50% levels found in the heterozygous knockout mice [17,19]. If these changes are largely mediated through haplotypes in the μ-opioid receptor gene or in genes that act to regulate it, such variants could contribute to the polygenic influences on human differences in various aspects of drug reward, and, conceivably, to human individual differences in addiction vulnerability.

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Methamphetamine and Brain Histamine

A Study Using Histamine-Related Gene Knockout Mice

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ABSTRACT: The central histamine (HA) neurons that originate from the posterior hypothalamus modulate a variety of physiological functions. In order to investigate the roles of brain histaminergic neuron system in the behavioral effects of methamphetamine (METH), we administrated METH repeatedly to L-histidine decarboxylase (HDC)–, histamine H1 receptor–, H2 receptor–gene knockout (KO) mice, H1/H2 receptor–gene double KO mice, and wild type (WT) mice corresponding to each of them, and we measured locomotor activities. We also measured the contents of monoamines and amino acids in the brain of HDC-gene KO and WT mice after a single administration of METH. METH-induced locomotor hyperactivity and the development of behavioral sensitization were facilitated more in the HDC-gene KO mice and H1/H2 gene double KO mice than the WT mice, suggesting that brain histamine has an inhibitory effect on the METH action through both H1 and H2 receptors. In addition, neurochemical study suggested the involvement of the GABAergic neuron system in the inhibitory effect of brain histamine.

KEYWORDS: histamine; methamphetamine; knockout mouse; behavioral sensitization

INTRODUCTION

Histamine (HA) has been recognized as an important neurotransmitter or neuromodulator in the central nervous system (CNS), which regulates various brain functions, such as circadian rhythm, feeding behavior, emotions, learning, and stress.[1,2] Synthesis of HA involves the transport of L-histidine into neurons and its single step of decarboxylation by L-histidine decarboxylase (HDC).

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