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Disruption of dopamine D_1 receptor gene expression attenuates alcohol-seeking behavior

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Abstract

The role of the dopamine D_1 receptor subtype in alcohol-seeking behaviors was studied in mice genetically deficient in dopamine D_1 receptors ($D_1-/-$). In two-tube free choice limited (1–5 h) and continuous (24 h) access paradigms, mice were exposed to water and increasing concentrations of ethanol (3%, 6% and 12% w/v). Voluntary ethanol consumption and preference over water were markedly reduced in $D_1-/-$ mice as compared to heterozygous ($D_1+/-$) and wild-type ($D_1+/+$) controls, whereas overall fluid consumption was comparable. When offered a single drinking tube containing alcohol as their only source of fluid for 24 h, $D_1-/-$ mice continued to drink significantly less alcohol than $D_1+/+$ and $D_1+/-$ mice. Dopamine D_2 receptor blockade with sulpiride caused a small but significant reduction in alcohol intake and preference in $D_1+/+$ mice and attenuated residual alcohol drinking in $D_1-/-$ mice. Dopamine D_1 receptor blockade with SCH-23390 very effectively reduced alcohol intake in $D_1+/+$ and $D_1+/-$ mice to the level seen in untreated $D_1-/-$ mice. These findings suggest involvement of both dopamine D_1 and D_2 receptor mechanisms in alcohol-seeking behavior in mice; however, these implicate D_1 receptors as having a more important role in the motivation for alcohol consumption. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Dopamine D_1 receptor-deficient mouse; Motivation; Reward; Alcohol drinking; SCH-23390; Sulpiride

1. Introduction

Dopamine is an important brain neurotransmitter that mediates a variety of functions such as locomotion and reward-related behaviors through activation of the mesolimbic and nigrostriatal systems. The rewarding and reinforcing properties of many substances of abuse including alcohol have been shown to be mediated in part by activation of the mesolimbic dopaminergic system (Di Chiara and Imperato, 1988; Kornetsky et al., 1988). Voluntary oral ingestion or acute intraperitoneal administration of ethanol has been shown to stimulate dopamine release selectively in the nucleus accumbens of ethanol-preferring rats (Yoshimoto et al., 1992; Weiss et al., 1993), as well as other rat strains unselected for ethanol preference (Imperato and Di Chiara, 1986). Moreover, ethanol has also been shown to activate dopamine neuronal firing in the ventral tegmental area (Gessa et al., 1985; Brodie et al., 1990). We have determined for a genetically inbred strain of mice that hypodopaminergic function in the mesolimbic system promotes alcohol intake (Ng et al., 1994b), which is attenuated by increasing synaptic dopamine (George et al., 1995). In addition, dopaminergic agonists were shown to reduce ethanol intake (Rassnick et al., 1993; Ng and George, 1994a; George et al., 1995). Although the available evidence suggests a positive relationship between stimulation of dopaminergic neurotransmission and the

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susceptibility to abuse alcohol, the mechanisms by which dopamine mediates alcohol abuse are still not clear.

Dopamine actions are mediated by two principal classes of receptor subtypes termed D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors (O'Dowd et al., 1994). While studies supporting the notion that dopamine plays a role in reward-related processes are well-documented, the relative contributions of different dopamine receptor subtypes in mediating the rewarding properties of ethanol are still controversial. Some have reported a lack of effect of dopamine D_2 receptor antagonists on alcohol drinking in a continuous access paradigm (Brown et al., 1982), whereas others have reported that dopamine D_2 receptor antagonists reduced operant responding for ethanol in a limited access paradigm as well as home cage alcohol drinking in rats (Pfeffer and Samson, 1986; Linseman, 1991; Rassnick et al., 1992; Dyr et al., 1993). In yet other studies, injection of dopamine D_2 receptor antagonist into the nucleus accumbens was reported to increase (Levy et al., 1991) and decrease (Samson et al., 1993) alcohol drinking. The involvement of dopamine D_1 receptors in mediating ethanol reward has also been documented (Kornetsky et al., 1988; Pfeffer and Samson, 1988; Dyr et al., 1993; Rassnick et al., 1993; Ng and George, 1994a; George et al., 1995; Hodge et al., 1997). These studies, although controversial, implicate important contribution of dopamine receptors in alcohol self-administration. However, the pharmacological agents so far available lack full selectivity for individual dopamine receptor subtypes which make it difficult to identify specific single candidate gene(s) that may predispose to alcoholism.

Given the established importance of the dopaminergic system in promoting alcohol consumption and mediating alcohol effects, we used dopamine D_1 receptor-deficient mice generated by homologous recombination (Drago et al., 1994) to investigate the role of this receptor in the motivation for alcohol drinking.

2. Materials and methods

2.1. Animals

The $D_1 -/-$ mice were generated and genotyped as previously reported (Drago et al., 1994). Three groups of adult male mice, derived from heterozygous mating, were used. These include homozygous mutant $D_1 -/-$, normal wild-type $D_1 +/+$ and heterozygous $D_1 +/-$ littermates. All mice were 4 months old and group-housed in a temperature-controlled room (22°C) maintained on a reversed 12 h dark–light cycle. All mice were given free access to food and water in their home cages. In addition to food pellets, mutant mice were given hydrated mouse chow meal (mash) at weaning age. Prior to the start of the experiment, the mash supplement was discontinued and all mice were fed the regular food pellets. Animal care was according to guidelines approved by the Canadian Council for Animal Care (CCAC).

2.2. Alcohol drinking

Initially, all mice were deprived of water for approximately 12 h after which they were transferred to individual stainless steel cages with wire mesh floor and were conditioned for 1 h per day for 3 days, to drink water from two graduated tubes adjusted to the front of the cage. All sessions were conducted during the dark phase of the dark–light cycle. On subsequent daily sessions, to test for alcohol preference, mice were not water- or food-deprived and were offered a two-tube free choice between alcohol and water in a limited access paradigm (1 h). The alcohol concentrations given were 3% w/v for 6 days, followed by 6% w/v for 7 days and finally 12% w/v which was maintained throughout the duration of the experiment. The positions of the tubes were reversed daily; alcohol and water consumption were recorded daily at the end of each drinking session; following which all mice were transferred to their home cages. After establishing a baseline level of 12% ethanol consumption, the access paradigm was changed to a 24-h continuous access once every 4 to 6 days. Alcohol and water consumption were recorded at 3, 4, 5 and 24 h and expressed as gram per kilogram and milliliter per kilogram body weight, respectively, with the percentage alcohol preference expressed as volume of alcohol consumed per total fluid volume (alcohol and water) consumed $\times$ 100.

2.2.1. Forced alcohol drinking

All mice were given three consecutive sessions in which they were offered only a single drinking tube containing 12% ethanol as their only source of fluid for 24 h.

2.3. Blood ethanol analysis

Naive mice were used for measurement of blood ethanol concentration to determine whether there were any inherent differences in the metabolism of ethanol among the three genotypes. From the cut tip of the mouse's tail, 20 μl mixed capillary and venous blood samples were collected at 0.5, 1, 1.5, 2, 3, 4 and 5 h after an intraperitoneal (i.p.) injection of ethanol 2.5 g/kg (12.5% w/v). The samples were deproteinized and analyzed as described previously (Hawkins et al., 1966).

2.4. Drug treatment

After acquiring a steady alcohol intake, all mice were habituated to receive saline injections prior to drinking sessions, then on subsequent sessions were subjected to saline or drug treatments before being offered free-choice

access to 12% w/v ethanol and water. Drug treatments were spaced apart by at least 2 weeks, during which basal alcohol intake was re-established. Drugs used were selegiline (*R*(−)-deprenyl HCl), 10 and 20 mg/kg; (−) sulpiride, 50 mg/kg and SCH-23390, 1 mg/kg. All chemicals were obtained from Research Biochemicals Int. (RBI, Natick, MA). Drugs were dissolved in 0.9% saline, with the exception of sulpiride which was dissolved in saline containing dilute acid, and injected subcutaneously (s.c.) in a volume of 1 μl/g body weight, 15–30 min prior to drinking sessions. All dose selections were based on our previous study (George et al., 1995).

2.5. Basal levels of dopamine and its metabolites

Naive mice from each genotype were killed by decapitation and the brains were quickly removed. Brain regions including the striatum, olfactory tubercle, midbrain, frontal cortex, medulla pons, hypothalamus and hippocampus were dissected on a chilled glass plate and stored at −70°C until analyzed by high performance liquid chromatography for biochemical measurements of dopamine and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), as described previously (George et al., 1995).

2.6. Receptor autoradiography

To confirm the effectiveness of dopamine D_1 receptor deletion and to assess dopamine D_2 receptor densities, naive mice from each genotype were sacrificed by decapitation and the brains removed, frozen in powdered dry ice and stored at −70°C until use. Frozen 12 μm coronal sections, from the anterior part of the corpus callosum to the substantia nigra, were cut on a cryostat and thaw-mounted onto slides and kept at −70°C for 1–2 days, after which they were dried for 15 min at room temperature. Slides were incubated in 50 mM Tris–HCl buffer (pH 7.4) containing 5 mM EDTA, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl and 120 mM NaCl. Autoradiographic dopamine D_1 receptor binding was carried out using 0.5 nM and 1.0 nM radiolabelled [^{3}H]SCH-23390 (NEN, Boston, MA) (specific activity = 71.3 Ci/mol), with 2 μM (+)-butaclamol (RBI, Natick, MA) to define non-specific binding. The slides were incubated in [^{3}H]SCH-23390 for 90 min at room temperature, then washed twice (10 min each) in ice-cold buffer, quickly dipped in ice-cold deionized water, dried and exposed to Hyperfilm (Amersham, Cleveland, OH) for 10 days at 4°C. Similarly, 1 nM [^{3}H]spiperone (NEN, Boston, MA) (specific activity = 15 Ci/mol), was

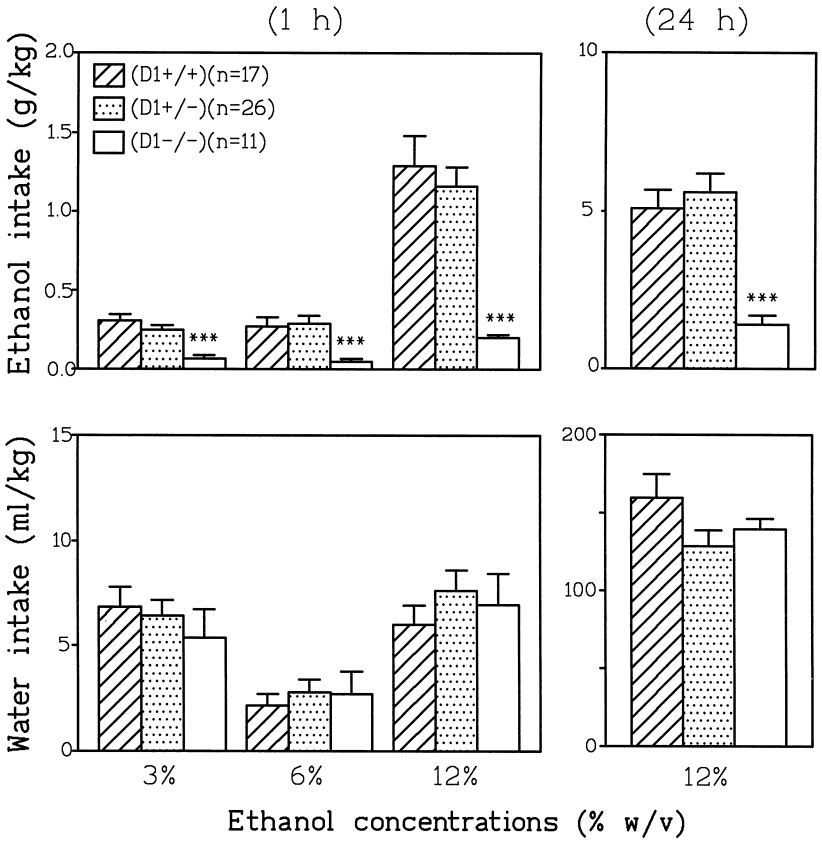

Fig. 1. Average ethanol intake (g/kg) and water intake (ml/kg) during 1 h free-choice access to 3%, 6%, and 12% w/v ethanol and water and 24 h access to 12% w/v ethanol and water on consecutive sessions. ANOVA detected a significant effect of genotype on ethanol intake [$F(2,51) = 11.45$, $P < 0.0001$; $F(2,51) = 4.73$, $P < 0.01$; $F(2,53) = 7.38$, $P < 0.001$] for 3%, 6%, and 12%, respectively, during 1 h access and for 12% during 24 h access [$F(2,51) = 12.45$, $P < 0.0001$]. Data shown are mean values ± S.E.M. ***, significantly different from $D_1+/+$ and $D_1+/-$, $P < 0.001$.

used for dopamine D_2 receptor binding with film exposure for 19–21 days.

2.7. Statistical analysis

Daily individual intakes of alcohol and water were recorded across sessions (baseline intake) and after each drug treatment and averaged for each group to obtain the mean ± S.E.M. One-way analysis of variance (ANOVA) was used to examine the effect of genotypes. Interactions between genotype and treatments were assessed by repeated measures of ANOVA. All analyses were followed by post-hoc Duncan's range tests to determine statistical significance ($P < 0.05$).

3. Results

The $D_1 -/-$ mice were smaller (20–30%) than $D_1 +/+$ or $D_1 +/-$ littermates; however, their overt home cage behavior as well as their spontaneous locomotor activity appeared normal and have been reported previously (Drago et al., 1994).

3.1. Alcohol drinking

When given free-choice access, $D_1 -/-$ mice had significantly lower alcohol intake ($P < 0.0001$) and preference ($P < 0.0001$) over water as compared to $D_1 +/+$ and $D_1 +/-$ mice. These differences were present across the various alcohol concentrations used and were evident during both limited and 24-h continuous access paradigms (Fig. 1). Water intake was comparable among all genotypes (Fig. 1).

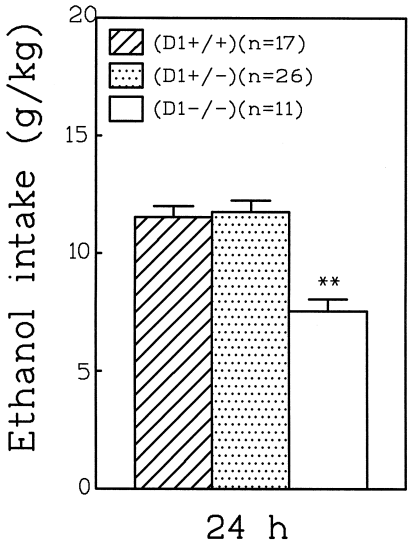

Fig. 2. Average ethanol intake (g/kg) during 24 h access to 12% w/v ethanol with no water available. All mice were injected with saline before exposure to alcohol. ANOVA detected a significant effect of genotype ($F(2,52) = 6.93$, $P < 0.002$) on alcohol intake, indicating that $D_1 -/-$ mice continued to maintain a significantly lower level of alcohol drinking than control groups. Data shown are means ± S.E.M. **, significantly different from $D_1 +/+$ and $D_1 +/-$, $P < 0.01$.

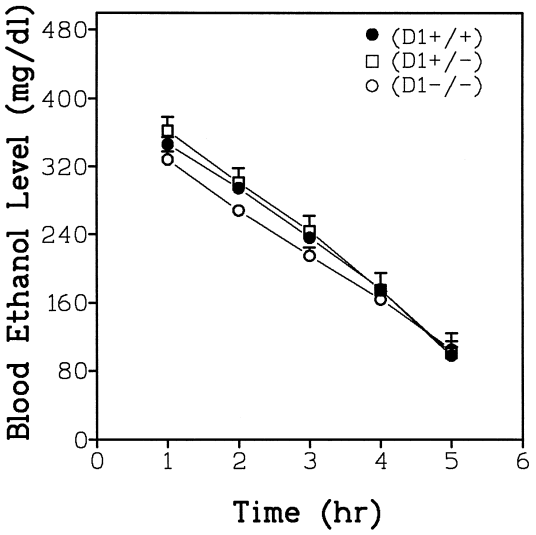

Fig. 3. Blood ethanol levels detected in mice ($n = 5$ per genotype group) at hourly intervals following an i.p. injection of ethanol 2.5 g/kg (12.5% w/v). ANOVA detected no significant effect of genotype ($F(2,48) = 2.37$, $P > 0.14$). Data shown are mean values ± S.E.M.

3.1.1. Forced alcohol drinking

When mice were offered a 12% ethanol solution as their only source of fluid for 24 h, the average alcohol intake was higher in all groups as compared to the free-choice intake; however, $D_1 -/-$ mice continued to drink significantly ($P < 0.01$) less alcohol than $D_1 +/+$ and $D_1 +/-$ mice in spite of correction for the lower body mass (Fig. 2).

3.2. Blood alcohol levels and metabolism

There was no significant difference in blood alcohol levels or rate of metabolism among any of the genotypes over 5 h following parenteral administration of ethanol (2.5 g/kg) (Fig. 3).

3.3. Effect of selegiline

In order to confirm that dopamine mediates alcohol drinking and preference, mice were treated with selegiline (10 and 20 mg/kg), a selective irreversible monoamine oxidase B inhibitor to block the oxidative deamination of dopamine, thereby increasing its synaptic levels. As compared to saline treatment, selegiline (10 mg/kg) reduced alcohol intake and preference in $D_1 +/+$ ($P < 0.05$) and $D_1 +/-$ ($P < 0.005$) mice over 3–5 h (Fig. 4); however, it had no effect in $D_1 -/-$ mice. At a higher dose (20 mg/kg), selegiline produced more marked reductions in

alcohol intake in all mice (data not shown). Water consumption was comparable across all groups (Fig. 4).

3.4. Effects of dopamine receptor antagonists

The involvement of dopamine D_1 and D_2 receptors in mediating alcohol drinking was further assessed by examining the effect of dopamine D_2 receptor antagonist (sulpiride) and dopamine D_1 receptor antagonist (SCH-23390) on voluntary intake of 12% ethanol in all groups.

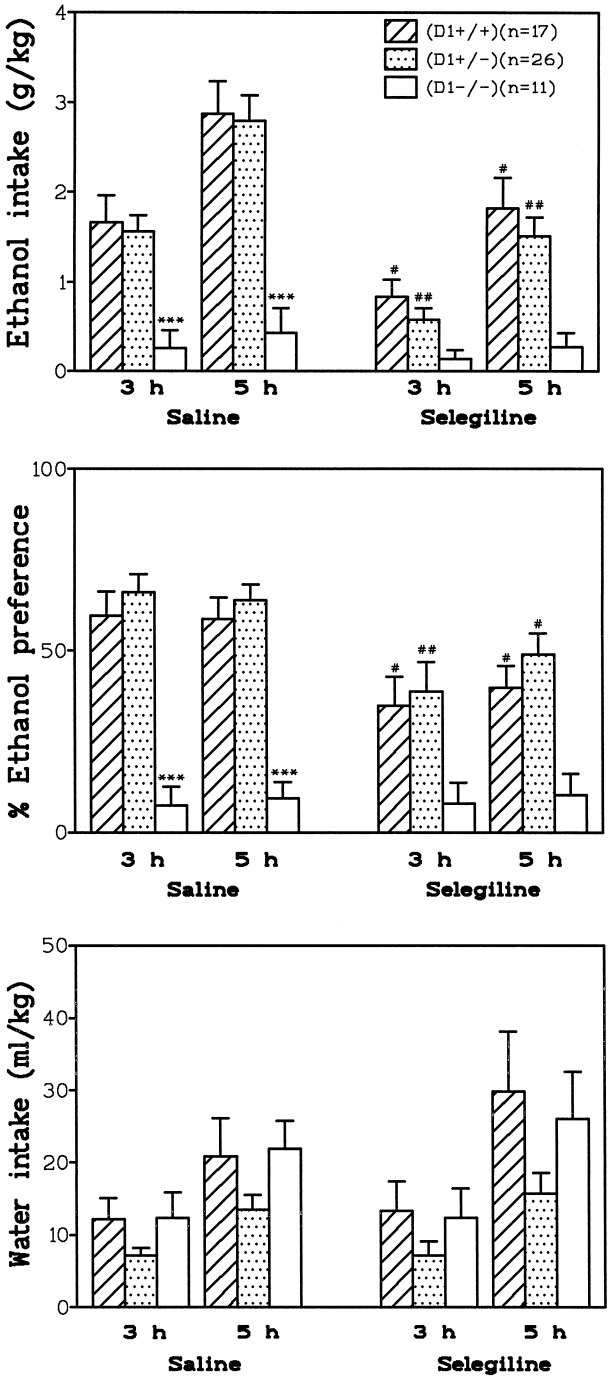

Fig. 4. Effect of selegiline (10 mg/kg) on average ethanol intake (g/kg), percentage ethanol preference and water intake (ml/kg). Mice were injected with either saline or selegiline in separate sessions and provided free-choice access to 12% w/v ethanol and water. Alcohol intake and preference over 3–5 h were not altered in $D_1-/-$ mice but were markedly reduced in $D_1+/+$ and $D_1+/-$ mice, following selegiline treatment as compared to saline. Data shown are mean values ± S.E.M. ANOVA at 3 h revealed a significant effect of genotype ($F(2,51)=7.19$, $P<0.002$) and treatment ($F(2,51)=16.43$, $P<0.0002$) on alcohol intake as well as a significant effect of genotype ($F(2,51)=13.78$, $P<0.00001$) and treatment ($F(2,51)=4.93$, $P<0.03$) on alcohol preference. There was no significant interaction between genotype and treatment, indicating that following selegiline treatment, $D_1-/-$ mice continued to maintain a significantly lower level of alcohol drinking and preference than control groups. Similar ANOVA results were obtained at 5 h. (***) Significantly different from $D_1+/+$ and $D_1+/-$, $P<0.0001$. (#), (##) Significantly different from saline treatment, $P<0.05$.

3.4.1. Effect of sulpiride

Sulpiride, at a dose (50 mg/kg) that did not cause any overt motor impairment, significantly attenuated (~80%, $P<0.03$) residual alcohol drinking and preference (~75%, $P<0.01$) in $D_1-/-$ mice, and caused small but significant reductions in alcohol consumption (~35%, $P<0.05$) and preference (~35%, $P<0.002$) in $D_1+/+$ mice over 3 h access as compared to basal levels following saline treatment (Fig. 5). Although sulpiride significantly ($P<0.01$) reduced alcohol preference in $D_1+/-$ mice, alcohol consumption was not significantly altered in these mice (Fig. 5). Water intake was not reduced by sulpiride treatment in any group over 3 h (Fig. 5).

3.4.2. Effect of SCH-23390

The SCH-23390, at a performance sparing dose (1 mg/kg), had no effect on $D_1-/-$ mice; however, it caused up to 75% reduction in alcohol intake in $D_1+/+$ ($P<0.0001$) and $D_1+/-$ ($P<0.002$) mice over 3 h access, as compared to basal levels following saline treatment. Alcohol preference was significantly reduced in $D_1+/-$ mice ($P<0.02$) but not in $D_1+/+$ mice (Fig. 6). Water consumption was not significantly altered in all mice (Fig. 6).

3.5. Dopamine turnover

To further investigate the possible development of adaptive changes in the dopaminergic system as a result of dopamine D_1 receptor deletion, biochemical measurements of dopamine and its metabolite, DOPAC, in certain regions of the brain were performed in vitro. There was a significant difference in dopamine and DOPAC levels among the genotypes in certain selected regions of the brain. In midbrain, $D_1-/-$ mice had significantly higher dopamine levels ($P<0.002$) than $D_1+/+$ mice, as well as higher DOPAC ($P<0.04$) levels in the medulla pons

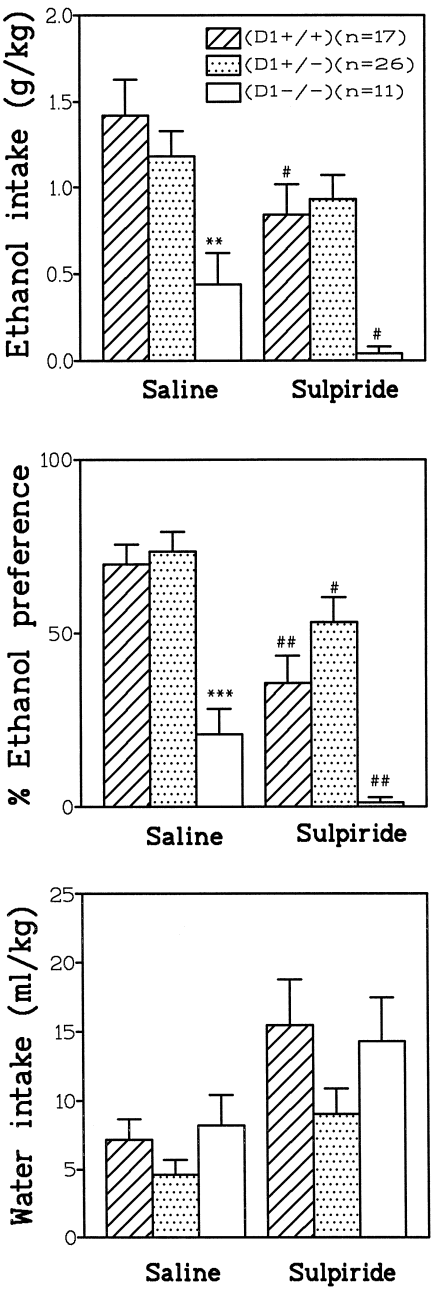

Fig. 5. Effect of sulpiride (50 mg/kg) on average ethanol intake (g/kg), percentage ethanol preference and water intake (ml/kg) during 3 h free-choice access to 12% ethanol and water. Mice were injected with either saline or sulpiride prior to drinking sessions. Alcohol intake and preference were reduced slightly (35%) in $D_1+/+$ but greatly (80%) in $D_1-/-$ mice after sulpiride treatment. ANOVA indicated a significant effect of genotype ($F(2,51)=3.92$, $P<0.03$) and treatment ($F(2,51)=17.72$, $P<0.0001$), on alcohol intake as well as a significant effect of genotype ($F(2,51)=10.02$, $P<0.0002$) and treatment ($F(2,51)=35.52$, $P<0.00001$) on alcohol preference. There was no significant genotype and treatment interaction on alcohol intake or preference, indicating that following sulpiride treatment, $D_1-/-$ mice continued to maintain a significantly lower level of alcohol drinking and preference than control groups. Data shown are mean values ± S.E.M. **, *** significantly different from $D_1+/+$ and $D_1+/-$, $P<0.01$. #, ## Significantly different from saline treatment, $P<0.05$.

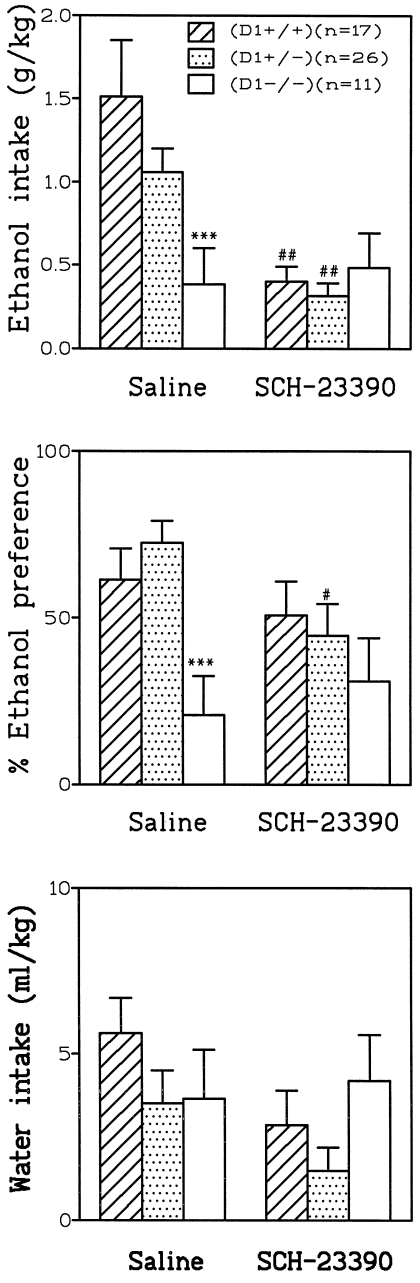

Fig. 6. Effect of SCH-23390 (1 mg/kg) on average ethanol intake (g/kg), percentage ethanol preference and water intake (ml/kg). Mice were injected with either saline or SCH-23390 and provided a free-choice access to 12% ethanol and water for 3 h. As compared to saline, SCH-23390 caused marked reductions in alcohol intake in $D_1+/+$ and $D_1+/-$ mice, but had no effect on $D_1-/-$ mice. ANOVA detected no significant effect of genotype but a significant effect of treatment ($F(2,50)=20.43$, $P<0.00003$) and genotype and treatment interaction ($F(2,50)=6.19$, $P<0.004$) on alcohol intake, indicating that SCH-23390 reduced alcohol intake in $D_1+/+$ and $D_1+/-$ mice to the same level as in the untreated $D_1-/-$ mice but had no effect on $D_1-/-$ mice. There was a significant effect of genotype ($F(2,50)=3.78$, $P<0.03$) and treatment ($F(2,50)=4,74$, $P<0.03$) but no significant effect of treatment or genotype and treatment interaction on alcohol preference. Data shown are mean values ± S.E.M. ***, significantly different from $D_1+/+$ and $D_1+/-$, $P<0.001$. #, ## significantly different from saline treatment, $P<0.05$.

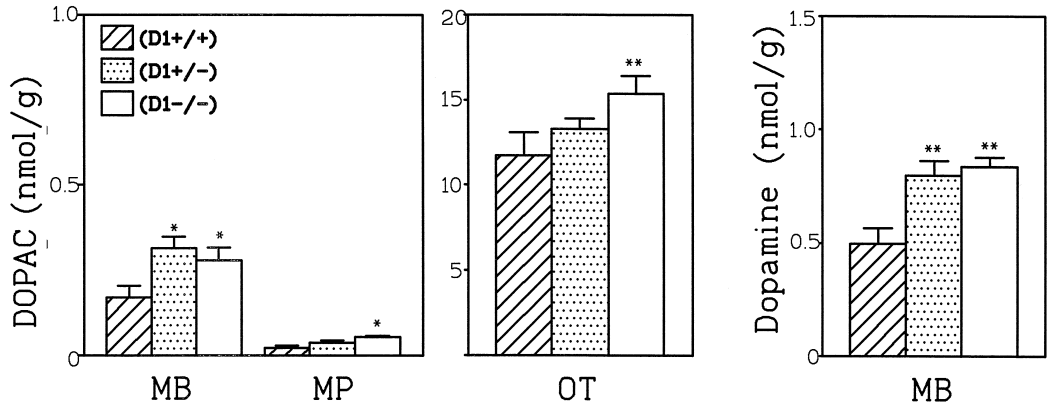

Fig. 7. Concentrations of DOPAC and dopamine in various brain regions of ethanol-naive $D_1+/+$, $D_1+/-$ and $D_1-/-$ mice ($n=6$ per group). The $D_1-/-$ mice had higher DOPAC levels in the midbrain (MB), medulla pons (MP) and olfactory tubercle (OT) and higher dopamine levels in MB than $D_1+/+$ mice. No parallel differences were observed between $D_1-/-$ and $D_1+/-$ mice. Values represent mean ± S.E.M. *, **, significantly different from $D_1+/+$, $P<0.05$.

and olfactory tubercle regions ($P<0.003$) (Fig. 7). The $D_1+/-$ mice also had higher levels of dopamine and DOPAC than $D_1+/+$ mice ($P<0.02$) in the midbrain region. There were no differences among genotypes in dopamine or DOPAC levels in other brain regions including the hippocampus, hypothalamus, frontal cortex, or striatum. Dopamine turnover rate, as reflected by the DOPAC to dopamine ratio, was significantly higher in $D_1-/-$ than $D_1+/+$ mice in medulla pons (+52%, $P<0.03$) and in olfactory tubercle (+29%, $P<0.001$). No differences in dopamine turnover rate were detected in other selected brain regions such as midbrain, hypothalamus, hippocampus, frontal cortex and striatum.

3.6. Receptor densities

Quantitative autoradiography for dopamine D_1 and D_2 receptor densities and distributions were performed in vitro. Autoradiographic data, in which [^{3}H]SCH-23390 binding sites correspond to dopamine D_1 receptors, detected no specific binding in frontal cortex, nucleus accumbens, olfactory tubercle, caudate putamen, subthalamic nucleus, suprachiasmatic nucleus, entopeduncular nucleus, and substantia nigra in $D_1-/-$ mice as compared to $D_1+/+$ mice; thus confirming the absence of dopamine D_1 receptors in the mutant mice. In these same regions, ~50% less dopamine D_1 receptor binding sites were

Table 1
Dopamine D_1 receptor density in various brain regions in ethanol-naive $D_1+/+$, $D_1-/-$ and $D_1+/-$ mice

Genotype	FC	NAc		OT	CP			STN	SCN	EN	SN
		Anterior	Posterior		Anterior	Medial	Posterior				
($D_1+/+$)	20 ± 2.2	296 ± 40	341 ± 16	309 ± 29	374 ± 14	375 ± 18	196 ± 29	50 ± 5.5	46 ± 8	91 ± 9.4	144 ± 14
($D_1-/-$)	7 ± 2.8[a]	7 ± 1.3[a]	7 ± 1.8[a]	4 ± 2.4[a]	2 ± 1.4[a]	5 ± 1.6[a]	8 ± 2.3[a]	4 ± 2.5[a]	8.4 ± 3[a]	4 ± 2.8[a]	9.4 ± 3.9[a]
($D_1+/-$)	13 ± 1.6	169 ± 9	141 ± 4	143 ± 25	178 ± 8	164 ± 6.8	114 ± 10	29 ± 3.8	16 ± 6.4	45 ± 3.9	68 ± 5

[^{3}H]SCH-23390 (1 nM) was used for D_1 receptor binding. Values are expressed as fmol/mg of tissue ± S.D.
[a] Significantly different from $D_1+/+$, $P<0.0001$.
FC = frontal cortex. NAc = nucleus accumbens. OT = olfactory tubercle. CP = caudate putamen. STN = subthalamic nucleus. SCN = suprachiasmatic nucleus. EN = entopeduncular nucleus. SN = substantia nigra.

Table 2
Dopamine D_2 receptor density in various brain regions in ethanol-naive $D_1+/+$, $D_1-/-$ and $D_1+/-$ mice

Genotype	FC	NAc		OT	CP			MN	VTA
		Anterior	Posterior		Anterior	Medial	Posterior		
$D_1+/+$	66 ± 8	81 ± 4	103 ± 5	136 ± 8	186 ± 2	216 ± 5	195 ± 7	18 ± 8	53 ± 4
$D_1-/-$	63 ± 9	86 ± 15	102 ± 7	144 ± 4	184 ± 1	211 ± 5	205 ± 8	19 ± 9	55 ± 7
$D_1+/-$	63 ± 3	85 ± 12	106 ± 6	141 ± 4	183 ± 3	206 ± 4	200 ± 5	20 ± 2	53 ± 6

[^{3}H]spiperone (1 nM) was used for D_2 receptor binding. Values are expressed as fmol receptor/mg of tissue ± S.D. No significant differences were observed among the groups. FC = frontal cortex. NAc = nucleus accumbens. OT = olfactory tubercle. CP = caudate putamen. MN = mammillary nucleus. VTA = ventral tegmental area.

detected in $D_1 +/-$ mice as compared to normal $D_1 +/+$ mice (Table 1). Dopamine D_2 binding sites labelled with 1 nM [^{3}H]spiperone indicated comparable dopamine D_2 receptor density in frontal cortex, nucleus accumbens, olfactory tubercle, mamilliary nucleus, ventral tegmental area and caudate putamen among all groups (Table 2).

4. Discussion

Since dopamine D_1 receptors have been shown to be involved in the rewarding effects of brain stimulation (Renaldi and Beninger, 1994) as well as in the rewarding and reinforcing effects of drugs of abuse, including alcohol (Dyr et al., 1993; Ng and George, 1994a; George et al., 1995; Hodge et al., 1997), it is conceivable that elimination of dopamine D_1 receptors would attenuate alcohol-seeking behavior. In the present study, we demonstrate clearly that $D_1 -/-$ mice have reduced alcohol drinking and preference as compared to $D_1 +/+$ and $D_1 +/-$ mice when offered a free-choice between alcohol and water in limited (1–5 h) as well as continuous (24 h) access paradigms. The difference in alcohol consumption between the mutant and normal mice was not related to differences in ethanol metabolism as demonstrated by the identical disappearance curves of blood ethanol following i.p. administration in all three genotypes. Furthermore, hunger due to non-availability of food for 5 h was not a stimulus for alcohol self-administration in these mice, since when food was provided during the entire drinking session, normal mice continued to drink significantly higher volumes of alcohol and showed higher preference for it as compared to the $D_1 -/-$ mice (data not shown). These results support the hypothesis that dopamine D_1 receptors may play a critical role in modulating alcohol drinking and that disruption of this single receptor gene can alter alcohol-seeking behavior.

It could be argued that the low level of alcohol drinking observed in $D_1 -/-$ mice is due to the fact that these animals never experienced the pharmacological and reinforcing effects of alcohol. Therefore, to test this hypothesis, all mice were forced to drink alcohol as their only available fluid for 24 h. The $D_1 -/-$ mice continued to drink significantly less alcohol than $D_1 +/+$ and $D_1 +/-$ mice. Although $D_1 -/-$ mice showed a slight increase in alcohol consumption and preference on free-choice access sessions afterward, which may suggest sensitization, they maintained levels significantly lower than controls and gradually reverted back to baseline levels. Since dopamine D_1 receptors have higher affinity for dopamine as compared to dopamine D_2 receptors, it is likely that low synaptic levels of dopamine released by low doses of ethanol consumed under free-choice paradigms mainly activate dopamine D_1 receptors. At higher doses of ethanol (forced drinking) where synaptic dopamine levels induced by ethanol are likely increased, the dopamine D_2-like receptors could be involved. Moreover, other neurotransmitter systems known to play a part in alcohol reward, such as 5-hydroxytryptamine (5-HT) (McBride et al., 1991; Crabbe et al., 1996), γ-aminobutyric acid (GABA) (Hyytia and Koob, 1995), glutamate (Rassnick et al., 1992) and the opioids (Froehlich et al., 1991) may contribute to alcohol drinking in these mutant mice. These findings clearly implicate a greater role of the dopamine D_1 receptor in alcohol-seeking behavior.

Evidence suggests that alcohol is reinforcing, in part, because of its effects on the mesolimbic dopaminergic system (Fibiger, 1993). Our present findings confirm our previous study (George et al., 1995) that elevation of synaptic dopamine levels by selegiline (10 and 20 mg/kg) via its dopamine enhancing effects may obviate the need to drink alcohol, thereby leading to reduced alcohol consumption in normal dopamine D_1 receptor expressing wild-type mice. Dopamine enhanced by selegiline is presumably capable of stimulating postsynaptic dopamine receptors. Recent evidence has indicated that selective dopamine D_1-like and D_2-like receptor agonists are self-administered (Self and Stein, 1992) and reduce the propensity for alcohol abuse (Rassnick et al., 1993; Ng and George, 1994a). This implies that activation of each receptor subtype may be rewarding. The fact that selegiline at a low dose (10 mg/kg) did not reduce alcohol intake further in $D_1 -/-$ mutants that have a normal expression of dopamine D_2 receptors (Table 2), and only a higher dose (20 mg/kg) was effective in attenuating residual alcohol intake by these mutants, may indicate that dopamine D_1 receptors are more important in mediating the reinforcing effects of alcohol. However, a lesser role of dopamine D_2 receptors also seems likely.

Although dopamine D_1 and D_2 receptor antagonists have been shown to modulate alcohol drinking and preference (Dyr et al., 1993; Samson et al., 1993; Panocka et al., 1995), the precise role of these specific receptor subtypes in alcohol reward has not been established. Our results show that dopamine D_2 receptor blockade by sulpiride caused a small (35%) but significant reduction in alcohol intake and preference in normal $D_1 +/+$ mice, whereas it attenuated residual alcohol intake (80%) and preference (75%) in $D_1 -/-$ mice bearing normal expression of dopamine D_2 receptors. Dopamine D_1 receptor blockade by SCH-23390 caused marked (75%) reduction in alcohol intake in $D_1 +/+$ and $D_1 +/-$ mice as compared to their basal intake following saline. In this situation, blocking the dopamine D_1 receptor in $D_1 +/+$ and $D_1 +/-$ mice reduced their alcohol intake to the same level as in the untreated $D_1 -/-$ mice, whereas the effect of blocking dopamine D_2 receptors in $D_1 +/+$ and $D_1 +/-$ mice was less marked. Our results are in agreement with the reported reduction in ethanol drinking in a limited access paradigm in ethanol-preferring rats following treatment with selective dopamine D_1 receptor antagonists, SCH-23390 (Dyr et al., 1993) and SCH-39166 (Panocka et

al., 1995). Alcohol preference was significantly reduced in $D_1+/-$ but not $D_1+/+$ mice following SCH-23390 treatment. This might be due to a slight but non-significant reduction in water intake in $D_1+/+$ mice.

In contrast to the effects observed with dopamine agonists (Dyr et al., 1993; Rassnick et al., 1993; Samson et al., 1993; Ng and George, 1994a) and elevation of dopamine levels by selegiline (George et al., 1995 and this study) which have been reported to reduce alcohol drinking, it might hypothetically be expected that dopamine D_1 or D_2 receptors antagonists would elevate alcohol drinking (analogous to the situation observed in dopamine antagonist-treated rats responding for intravenous psychomotor stimulants (Corrigall and Coen, 1991). However, our current and previously reported results indicate that this does not occur with systemically administered dopamine antagonists and suggest that blocking dopamine D_1 or D_2 receptors may attenuate the rewarding value of alcohol, or alternatively elevate endogenous dopaminergic activity and thus reduce the drive for alcohol drinking. In fact, many studies have indicated that antagonistic activity at dopamine D_1 and D_2 receptors increased dopamine synthesis and release (Imperato et al., 1987; See et al., 1991; Santiago et al., 1993).

On the basis of the larger effect of SCH-23390 vs. sulpiride in $D_1+/+$ mice, together with lower basal ethanol consumption and preference under free-choice and forced drinking paradigms, disruption of dopamine D_1 receptor function appears to have a greater impact on alcohol drinking than does dopamine D_2 receptor blockade. These findings clearly imply that both dopamine D_1 and D_2 receptors are involved in mediating alcohol-seeking behavior; however, dopamine D_1 receptors appear to have a more profound role.

Measurements of dopamine and its metabolite indicated that $D_1-/-$ mice had higher levels of DOPAC than $D_1+/+$ mice in several brain regions including midbrain, medulla pons and olfactory tubercle as well as a higher dopamine turnover rate in the medulla pons and olfactory tubercle. Furthermore, in the midbrain region, the $D_1-/-$ mutants had a higher level of dopamine than the normal $D_1+/+$ mice. Since the mutant mice showed no gross physiological or anatomical abnormality (Drago et al., 1994; Xu et al., 1994), one possible explanation is that compensatory mechanisms have developed to overcome the biochemical imbalance as a result of reduced dopaminergic function.

As revealed by autoradiography, our results confirm and extend our previous report (Drago et al., 1994) by documenting the absence of dopamine D_1 receptor binding in regions of the brain known to express these receptors. However, dopamine D_2 receptor density was comparable among all groups in frontal cortex, nucleus accumbens, olfactory tubercle, mamilliary nucleus, ventral tegmental area and caudate putamen.

This study clearly demonstrates an important role for the dopamine D_1 receptor gene in alcohol drinking and provides a clear example of a single gene disruption leading to attenuation of alcohol-seeking behavior. The role of the dopamine D_1 receptor in other forms of substance abuse remains to be investigated. Moreover, in light of the recent demonstration of 5-HT_{1B} receptor deletion augmenting alcohol intake (Crabbe et al., 1996), it would be of considerable interest to determine the relative importance of these two receptor systems in modulating alcohol-seeking behavior.

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References

Brodie, M.S., Shefer, S.A., Dunwiddie, T.V., 1990. Ethanol increases the firing of dopamine neurons of the rat ventral tegmental area in vitro. Brain Res. 508, 65–69.

Brown, Z.W., Gill, K., Abitbol, M., Amit, A., 1982. Lack of effect of dopamine receptor blockade on voluntary ethanol consumption in rats. Behav. Neurol. Biol. 36, 291–294.

Corrigall, W.A., Coen, K.M., 1991. Cocaine self-administration is increased by both D_1 and D_2 dopamine antagonists. Pharmacol. Biochem. Behav. 39, 799–802.

Crabbe, J.C., Phillips, T.J., Feller, D.J., Hen, R., Wenger, C.D., Lessov, C.N., Schafer, G.L., 1996. Elevated alcohol consumption in null mutant mice lacking 5-HT_{1B} serotonin receptors. Nature Genet. 14, 98–101.

Di Chiara, G., Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentration in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85, 5274–5278.

Drago, J., Gerfen, C.R., Lachowicz, J.E., Steiner, H., Hollon, T.R., Love, P.E., Ooi, G.T., Grinberg, A., Lee, E.J., Huang, S.P., Bartlett, P.F., Jose, P.A., Sibley, D.R., Westphal, H., 1994. Altered striatal function in a mutant mouse lacking D_{1A} dopamine receptors. Proc. Natl. Acad. Sci. USA 91, 12564–12568.

Dyr, W., McBride, W.J., Leumeng, L., Li, T.K., Murphy, J.M., 1993. Effects of D_1 and D_2 dopamine receptor agents on ethanol consumption in the high alcohol drinking (HAD) line of rats. Alcohol 10, 207–212.

Fibiger, H.C., 1993. Mesolimbic dopamine: an analysis of its role in motivated behavior. Neuroscience 5, 321–327.

Froehlich, J.C., Zweifel, M., Harts, J., Lumeng, L., Li, T.K., 1991. Importance of delta opioid receptors in maintaining high alcohol drinking. Psychopharmacology 103, 467–472.

George, S.R., Fan, T., Ng, G.Y.K., Jung, S.Y., O'Dowd, B.F., Naranjo, C.A., 1995. Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. J. Pharmacol. Exp. Ther. 273, 373–379.

Gessa, G.L., Muntoni, F., Collu, M., Vargiu, L., Mereu, G., 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. Brain Res. 348, 201–203.

Hawkins, R.D., Kalant, H., Khanna, J.M., 1966. Effects of chronic intake

of ethanol on rate of ethanol metabolism. Can. J. Physiol. Pharmacol. 44, 241–257.

Hodge, C.W., Samson, H.H., Chappelle, A.M., 1997. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. Alcohol. Clin. Exp. Res. 21, 1083–1091.

Hyytia, P., Koob, G.F., 1995. $GABA_A$ in the extended amygdala decreases ethanol self-administration in rats. Eur. J. Pharmacol. 283, 151–159.

Imperato, A., Di Chiara, G., 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 239, 219–228.

Imperato, A., Mulas, A., Di Chiara, G., 1987. The D_1 antagonist, SCH-23390, stimulates while D_1 agonist, SKF-38393, fails to affect dopamine release in the dorsal caudate of freely moving rats. Eur. J. Pharmacol. 142, 177–181.

Kornetsky, C., Bam, G.I., Unterwald, E.M., Lewis, M.J., 1988. Brain stimulation reward: effect of ethanol. Alcoholism 12, 609–616.

Levy, A.D., Murphy, J.M., McBride, W.J., Lumeng, L., Li, T.K., 1991. Microinjection of sulpiride into nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats. Alcohol Alcohol. 1, 417–420, Suppl.

Linseman, M.A., 1991. Effects of dopaminergic agents on alcohol consumption by rats in a limited access paradigm. Psychopharmacology 100, 195–200.

McBride, W.J., Murphy, J.M., Lumeng, L., Li, T.K., 1991. Serotonin and dopamine systems regulating alcohol intake. Alcohol Alcohol. 1, 411–416, Suppl.

Ng, G.Y.K., George, S.R., 1994a. Dopamine receptor agonist reduces ethanol self-administration in ethanol-preferring (C57BL/6J) inbred mouse. Eur. J. Pharmacol. 269, 365–374.

Ng, G.Y.K., O'Dowd, B.F., George, S.R., 1994b. Genotypic differences in brain dopamine receptor function in the DBA/2J and C57BL/6J inbred mouse strains. Eur. J. Pharmacol. 269, 349–364.

O'Dowd, B.F., Seeman, P., George, S.R., 1994. G protein-coupled receptors. In: Peroutka, S.J. (Ed.), Handbook of Receptors and Channels. CRC Press, Boca Raton, FL, pp. 95–123.

Panocka, I., Ciccocioppo, R., Mosca, M., Polidori, C., Massi, M., 1995. Effects of the dopamine D_1 receptor antagonist, SCH-23166, on the ingestive behavior of alcohol-preferring rats. Psychopharmacology 120, 227–235.

Pfeffer, A.O., Samson, H.H., 1986. Effect of pimozide on home cage ethanol drinking in the rat: dependence on drinking session length. Drug Alcohol Depend. 17, 47–55.

Pfeffer, A.O., Samson, H.H., 1988. Haloperidol and apomorphine effects on ethanol reinforcement in free-feeding rats. Pharmacol. Biochem. Behav. 29, 343–350.

Rassnick, S., Pulvirenti, L., Koob, G.F., 1992. Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens. Psychopharmacology 109, 92–98.

Rassnick, S., Pulvirenti, L., Koob, G.F., 1993. SDZ-205152, a novel dopamine receptor agonist, reduces oral ethanol self-administration in rats. Alcohol 10, 127–132.

Renaldi, R., Beninger, R.J., 1994. The effects of systemic and intracerebral injection of D_1 and D_2 agonists on brain stimulation reward. Brain Res. 651, 283–292.

Samson, H.H., Hodge, C.W., Tolliver, G.A., Haraguchi, M., 1993. Effect of dopamine agonists and antagonists on ethanol-reinforced behavior, the involvement of the nucleus accumbens. Brain Res. Bull. 30, 133–141.

Santiago, M., Machado, A., Cano, J., 1993. Regulation of prefrontal cortical dopamine release by dopamine receptor agonists and antagonists. Eur. J. Pharmacol. 239, 83–91.

See, R.E., Sorg, B.A., Chapman, M.A., Kalivas, P.W., 1991. In vivo assessment of release and metabolism of dopamine in the ventrolateral striatum of awake rats following administration of dopamine D_1 and D_2 receptor agonists and antagonists. Neuropharmacology 30, 1269–1274.

Self, D.W., Stein, L., 1992. The D_1 agonists, SKF82958 and SKF77434, are self-administered by rats. Brain Res. 582, 349–352.

Weiss, F., Lorange, M.T., Bloom, F.E., Koob, G.F., 1993. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J. Pharmacol. Exp. Ther. 267, 250–258.

Xu, M., Moratalla, R., Gold, L.H., Hiroi, N., Koob, G.F., Graybiel, A.M., Tonegawa, S., 1994. Dopamine D_1 receptor mutant mice are deficient in striatal expression of dynorphin and in dopamine-mediated behavioral responses. Cell 79, 729–742.

Yoshimoto, K., McBride, W.J., Lumeng, L., Li, T.K., 1992. 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.