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ORIGINAL INVESTIGATION

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Lack of operant ethanol self-administration in dopamine D_2 receptor knockout mice

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Abstract *Rationale:* Dopamine D_2 receptors are postulated to play an important role in modulating the reinforcing effects of abused drugs including ethanol. *Objectives:* This experiment examined operant ethanol self-administration in dopamine D_2 receptor knockout (KO) mice and wild-type (WT) mice using a continuous access procedure. *Methods:* Adult male KO and WT mice were trained in 30-min sessions to perform a lever press response for access to 10% v/v ethanol. After training, the mice were placed in test chambers on a continuous (23 h/day) basis with access to food (one lever press, i.e., FR1), 10% v/v ethanol (four lever presses, i.e., FR4), and water from a sipper tube (phase 1). After 30 consecutive sessions, response patterns were determined for 0, 5, 10, 20 and 30% v/v ethanol (phase 2). Saccharin (0.2% w/v) was subsequently added to the ethanol mixture and responding was examined for 0, 5, 10 and 20% ethanol (phase 3). *Results:* During phase 1, WT mice displayed higher ethanol-lever responding compared to KO mice. Food lever responding and water intake was the same in both genotypes. During phase 2, WT mice displayed concentration-dependent ethanol lever responding, whereas KO mice responded at low rates regardless of ethanol concentration. WT mice also responded more for food compared to KO mice. Each genotype showed similar water intakes except at the 20% ethanol concentration, where WT mice had lower intakes. During phase 3, WT mice continued to show higher responding for all concentrations including saccharin alone. WT mice also continued to respond more for food compared to KO mice, but drank less water. In each phase, WT mice displayed episodic (bout) responding on the ethanol lever. KO mice did not respond for ethanol in bouts. *Conclusions:* Reduced responding in the KO mice for several reinforcers including ethanol indicates a more general role for dopamine D_2 receptors in motivated responding rather than a specific role in ethanol reinforcement.

Key words Ethanol self-administration · Reinforcement · Continuous access · Dopamine D_2 receptor knockout mice

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Introduction

Brain dopaminergic receptor systems are thought to be important for the rewarding effects of abused drugs including ethanol (Samson and Harris 1992; Koob and Nestler 1997; Koob et al. 1998; Wise 1998). One important dopaminergic pathway for drug reward appears to be mesocorticolimbic brain areas which include the ventral tegmental area, nucleus accumbens, olfactory tubercle, amygdala, frontal cortex and septal area (Koob 1992). Dopamine receptor subtypes have been classified as D_1-like (D_1 and D_5 receptors) and D_2-like (D_2, D_3, and D_4 receptors)(Civelli et al. 1993). Both D_1-like and D_2-like receptors are found in cortical brain areas thought to be important for drug reward (Civelli et al. 1993).

Pharmacological manipulations have been used to investigate the role of different dopamine receptor types in the mediation of ethanol reward or self-administration. Attention has often been drawn to dopamine D_2 receptors. Ethanol drinking determined in rats or mice using

two-bottle choice procedures is sensitive to both dopamine D_2 receptor antagonists and agonists. Ethanol preferring C57BL/6 mice demonstrated reduced ethanol intake after treatment with the D_2 receptor agonist bromocriptine and the D_2 receptor antagonist haloperidol (Ng and George 1994). In rats, the D_2 receptor antagonist pimozide reduced ethanol intake in one study (Hubbell et al. 1991). However, pimozide and the D_2/D_3 receptor antagonist raclopride did not affect ethanol consumption in two other studies (Goodwin et al. 1996; Silvestre et al. 1996). Further, in a limited access procedure, the D_2 agonist quinpirole decreased ethanol intake, and the D_2 antagonist spiperone increased ethanol intake (Dyr et al. 1993). D_2 receptor antagonists (e.g., haloperidol) have also been shown to reduce operant behavior for ethanol access in rats (Pfeffer and Samson 1988; Cohen et al. 1998). Haloperidol blocks ethanol-stimulated activity in mice but not the acquisition of ethanol-induced conditioned place preference (Risinger et al. 1992). Haloperidol also reduces acquisition of ethanol-induced conditioned taste aversion (Risinger et al. 1999a).

Recently, mutant mice lacking dopamine D_2 receptors have been developed (Kelly et al. 1997). These mice appear developmentally normal, but show reduced levels of locomotor activity in an open field test, and reduced ability to perform on the rotarod (Kelly et al. 1998). Striatal dopamine levels in KO mice do not differ from parental strains (i.e., C57BL/6, 129/SvEv) or WT mice, and the distribution of D_1 receptor binding sites is similar to WT mice (Kelly et al. 1998). KO mice drink less ethanol in a two-bottle home cage procedure compared to heterozygotes and WT mice on the C57BL/6 (C57) background (Phillips et al. 1998). These results compare favorably with the identification of a quantitative trait locus on mouse chromosome 9 near the dopamine D_2 gene that has been associated with ethanol consumption and preference in the home cage (cf. Phillips et al. 1994).

The present study was devoted to characterizing operant responding for ethanol in the D_2 KO mice. We postulated that KO mice would display substantially lower levels of ethanol self-administration compared to WT mice in a continuous access procedure utilizing 23-h/day sessions. In this procedure, C57 mice show concentration-dependent responding for access to ethanol on an FR4 schedule of reinforcement. DBA/2J (DBA) mice do not respond for ethanol over the level of responding seen for plain water (Risinger et al. 1998). Further, C57 mice demonstrate episodic responding for ethanol (i.e., bouts), whereas DBA mice do not.

Materials and methods

Subjects

The present study used male homozygous KO mice (–/–) in comparison to homozygous WT mice (+/+). Male incipient congenic mice (N10) on a C57BL/6J background were produced at Oregon Health Sciences University (Kelly et al. 1998). Animals were between 3–4 months old at the beginning of training. Prior to and during training, mice were housed two to four per cage in polycarbonate cages (27.9×9.5×12.7 cm) with cob bedding. The colony room was maintained on a normal 12-h light/dark cycle (lights on at 0700 hours) at an ambient temperature of 21±1°C. Lever response training was conducted during the light cycle. Lab chow was continuously available in the home cage. Daily access to fluids during training was restricted according to the procedure describe herein.

Apparatus

Lever response training was conducted in four mouse test chambers (Med Associates Modular Mouse Test Chamber, ENV-307A) each equipped with one ultra-sensitive mouse lever (Med Associates ENV-310), liquid dipper with a 0.02 ml cup (Med Associates ENV-303), and 100 mA house light. The house light was located on the opposite wall from the location of the lever and liquid dipper, and was on when a session was active. Each chamber was enclosed in a light/sound attenuating cubicle (Med Associates ENV-015M). For 23-h sessions, 15 mouse test chambers (Med Associates ENV-003) enclosed in light/sound attenuating cubicles were used. Each chamber was equipped with two ultra-sensitive mouse levers, liquid dipper with a 0.02 ml cup, 20 mg pellet dispenser (Med Associates ENV-203-20), drinking tube and house light. The access well for the liquid dipper was located in the center of the right side panel. The access well for the pellet dispenser was located in the center of the left panel. The levers were placed on the left side of the liquid dipper well and pellet dispenser. The drinking tube (25 ml glass graduated cylinder fitted with a stainless steel drinking spout) was located in the center of the front panel and connected to a contact lickometer (Med Associates ENV-250A). The house light was centered on the left side panel 9.5 cm above the floor. Session parameters and data collection were controlled by computers adjacent to the chambers using Med Associates interface modules.

Procedure

During training, subjects received 2-h access to water each day, 4 h after training sessions. Training and ethanol initiation sessions were 30 min in duration. Subjects were first trained to lever-press for 20% w/v sucrose solution. Initially, one lever press resulted in 10-s access to the dipper cup (i.e., FR1 schedule of reinforcement). During the course of a 10-day training phase, the schedule of reinforcement was gradually increased to FR4, and the dipper access period reduced to 5 s. When training was complete, the subjects entered a 10-day initiation phase during which an increasing concentration of ethanol was gradually introduced to the sucrose solution. The concentration of sucrose was gradually reduced such that at the end of this phase subjects were receiving access to 10% v/v ethanol in tap water. Water restriction was maintained during this phase in order to keep response rates reasonably high.

Following the initiation phase, subjects (n=8 KO, 7 WT) were placed in the test chambers for 23-h sessions. Initially, 10% v/v ethanol was available from the dipper (FR4), food from the pellet dispenser (20 mg Noyes Formula A pellets, FR1) and water from the drinking tube. Each day, subjects were removed from the chamber for 1 h in order to clean and resupply the chambers. A 12:12 h light/dark cycle was maintained throughout the procedure.

The first session was used for acclimation to the chambers and procedure, and data from this session were not subjected to analysis of strain differences. Subsequently, phase 1 consisted of 30 consecutive 23-h sessions with 10% v/v ethanol available. At the end of phase 1, the concentration of ethanol was changed every five sessions (designated as phase 2). The following v/v concentrations of ethanol were presented in the following order: 5, 10, 20, 30, 0. For phase 3, 0.2% w/v saccharin was used as the ethanol vehicle. The addition of the 0.2% saccharin was expected to increase overall ethanol consumption in both genotypes (cf. Risinger

et al. 1998). The % v/v concentration of ethanol was changed every five sessions in the following order: 0, 5, 10, 20.

Data analyses

Initial comparisons of genotype focused on daily session response rates for ethanol and food, and water intake. Further, a microanalysis of eating and drinking was conducted using a procedure that defined temporally related sequences of behavior as bouts (Samson et al. 1988). A food bout was defined as two or more pellet deliveries within 2 min or less. An ethanol bout consisted of four or more dipper presentations with 2 min or less between each dipper presentation. A water bout consisted of at least 25 consecutive licks with 2 min or less between each lick. The relationship between food intake, ethanol and water bouts was also determined (prandial bouts or non-prandial bouts). Prandial bouts occurred during or within 5 min of completion of a food bout. Non-prandial bouts occurred at least 5 min after the completion of a food bout. Unweighted means analysis of variance (ANOVA) was used for analysis. An alpha level of $P<0.05$ was used. For analyses based on repeated measures, probability determinations were based on a Greenhouse-Geisser correction for inflated alpha.

Results

Although both KO mice and WT mice acquired the lever press response during training, KO mice showed lower responses overall. For example, at the end of the 10-day training phase when all subjects were on an FR4 schedule of reinforcement for 20% sucrose, KO mice generated a mean of 17.5 (±4.6) responses in the 30-min session compared to 38.3 (±4.9) responses for the WT mice [$F(1,13)=9.5$, $P<0.009$]. This genotype difference continued such that at the end of the ethanol initiation phase WT mice responded more for 10% ethanol compared to KO mice [KO mice: 47.8±21.3 responses/session; WT mice: 138.3±22.7 responses/session; $F(1,13)=8.4$, $P<0.01$]. Based on the volume of fluid presented on the last session of initiation, KO mice received 1.1±0.3 g/kg ethanol compared to 1.7±0.3 g/kg for WT mice.

Figure 1, Fig. 2 and Fig. 3 depict ethanol lever responding, food lever responding, and water intake for phases 1–3, respectively. Table 1, Table 2 and Table 3 give total fluid consumption, ethanol bouts, water bouts and food bouts for each phase. Data from each subject were averaged over five session blocks. For phases 2 and 3, each subject's data were averaged over each ethanol concentration. During phase 1, WT mice consistently responded more on the ethanol lever than KO mice (Fig. 1). Repeated measures analysis (Genotype×Trial block) showed a significant effect of Genotype [$F(1,13)=47.4$, $P<0.001$] and Genotype×Trial Block [$F(5,65)=4.5$, $P<0.02$]. Follow-up analyses of each genotype over trial block indicated that although responding appeared to increase for WT mice and decrease for KO mice, these changes did not reach statistical significance. Food lever responding did not differ between genotypes and did not change over trial block. Analysis of water intake yielded significant effects of Trial block [$F(5,65)=14.8$, $P<0.001$] and Genotype×Trial block [$F(5,65)=3.8$, $P<0.02$], but not Genotype. Follow-up analyses of each genotype over trial block yielded significant trial block effects in both genotypes [KO: $F(5,35)=5.2$, $P<0.02$; WT: $F(5,30)=9.7$, $P<0.01$]. Significant effects of genotype were not seen at any trial block.

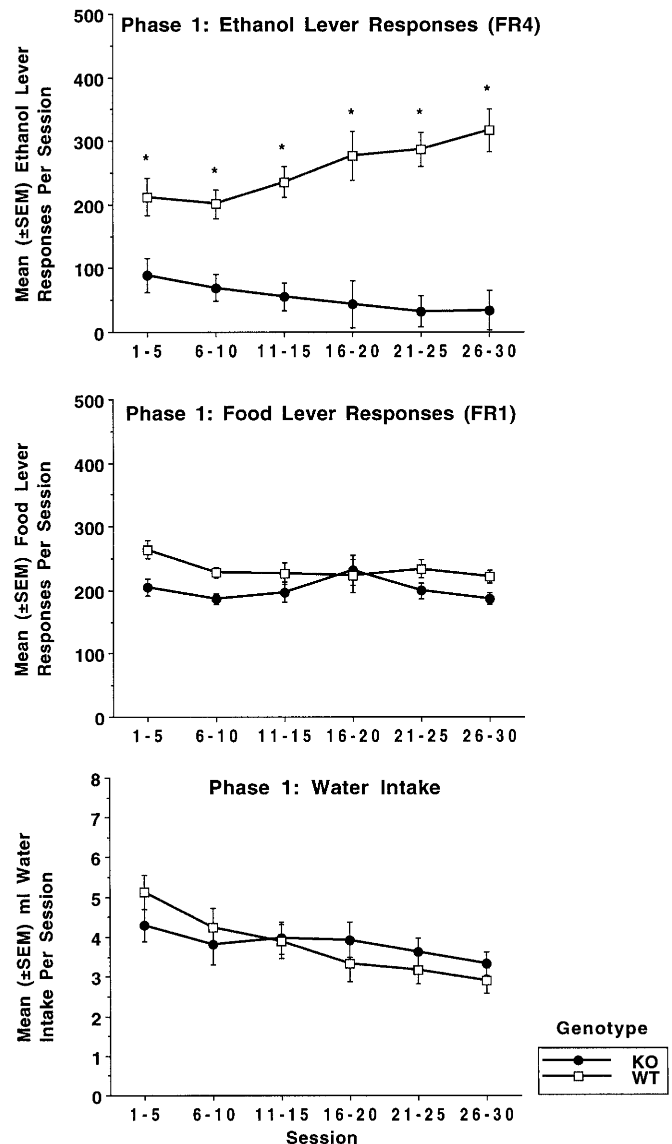

Fig. 1 Mean (±SEM) number of ethanol lever responses per session (*top panel*), food lever responses (*middle panel*), and ml water intake (*bottom panel*) for phase 1. Ethanol was presented on an FR4 schedule of reinforcement. Food was presented on an FR1 schedule of reinforcement. Significant Genotype effects (KO compared to WT, $P<0.05$) are noted with an *asterisk*

Analysis of total fluid consumed yielded a significant effect of Trial block [$F(5,65)=19.3$, $P<0.001$] and Genotype×Trial block [$F(5,65)=2.7$, $P<0.05$] (Table 1). Analysis of Trial block indicated significant declines in total fluid intake during phase 1 for both genotypes [KO: $F(5,35)=8.0$, $P<0.002$; WT: $F(5,30)=12.3$, $P<0.001$]. Genotype comparisons at each five-session block yielded significant Genotype effects for the first and last five-session block [both Fs$(1,13)>5.4$, Ps<0.04]. WT mice

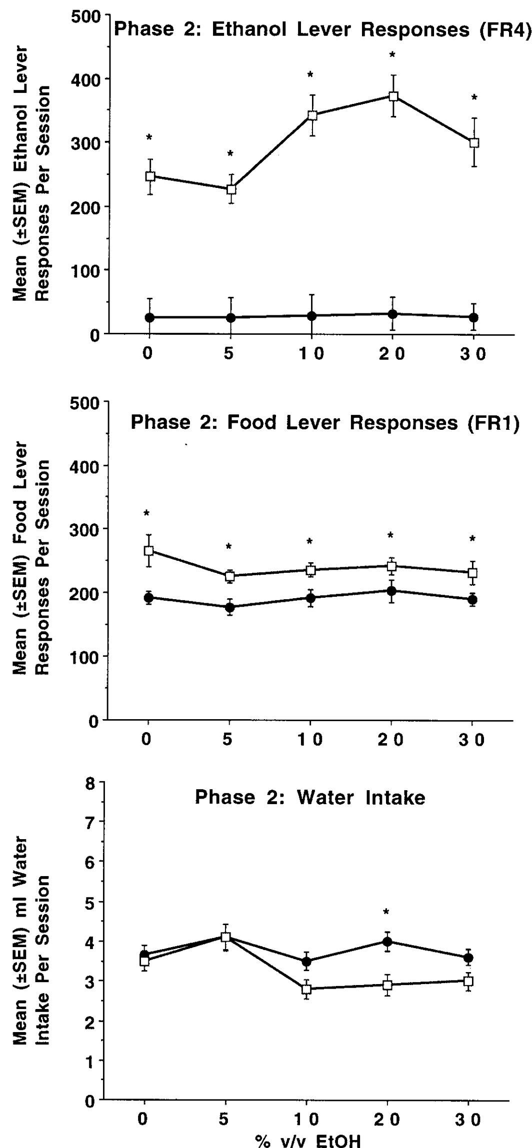

Fig. 2 Mean (±SEM) number of ethanol lever responses per session (*top panel*), food lever responses (*middle panel*), and ml water intake (*bottom panel*) for phase 2. Ethanol was presented on an FR4 schedule of reinforcement. Food was presented on an FR1 schedule of reinforcement. Significant Genotype effects (KO compared to WT, $P<0.05$) are noted with an *asterisk*

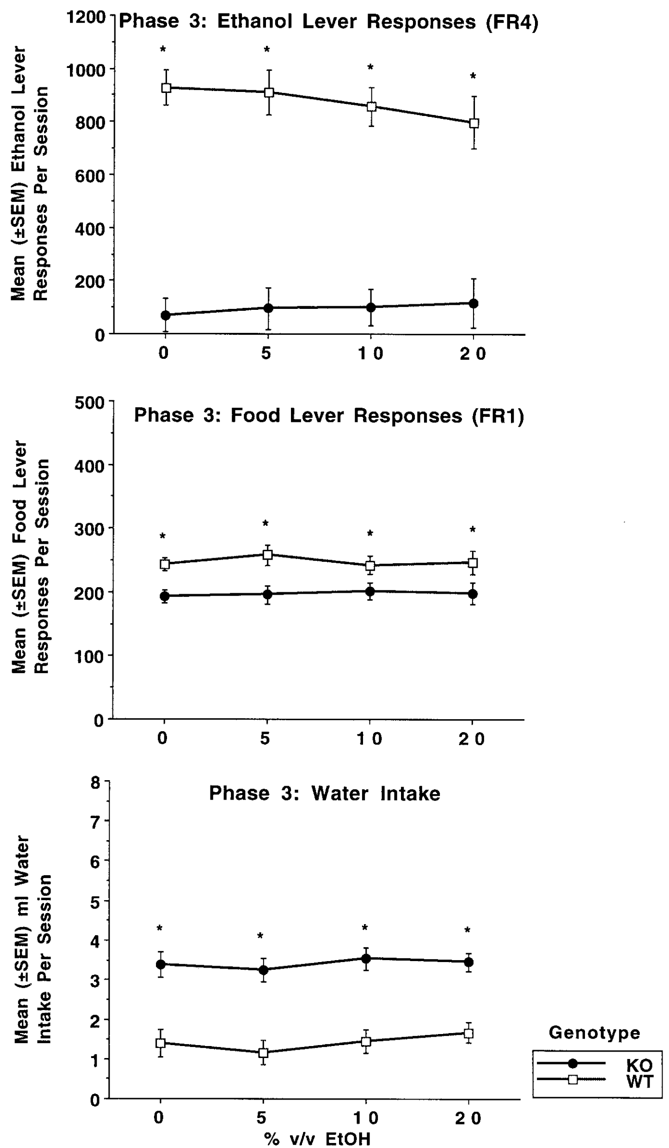

Fig. 3 Mean (±SEM) number of ethanol lever responses per session (*top panel*), food lever responses (*middle panel*) and ml water intake (*bottom panel*) for phase 3. Ethanol was presented on an FR4 schedule of reinforcement. Food was presented on an FR1 schedule of reinforcement. Significant Genotype effects (KO compared to WT, $P<0.05$) are noted with an *asterisk*

consistently generated ethanol bouts each session, whereas KO mice did not. Both genotypes showed similar frequencies of water bouts and food bouts.

During phase 2, WT mice showed concentration-dependent changes in responding on the ethanol lever (Fig. 2). Total responses in the KO mice did not change as a function of ethanol concentration. Further, total responses were lower across all ethanol concentrations in the KO mice compared to WT mice. Genotype×Ethanol concentration analysis yielded significant effects of Genotype [$F(1,13)=55.5$, $P<0.001$], Ethanol concentration [$F(4,52)=6.6$, $P<0.002$], and Genotype×Ethanol concentration [$F(4,52)= 5.6$, $P<0.005$]. Follow-up analysis of Ethanol concentration within each genotype yielded a significant effect of Ethanol concentration in WT mice [$F(4,24)=5.4$, $P<0.02$], but not in KO mice. Significant effects of Genotype were seen at each ethanol concentration [all Fs$(1,13)>28.1$, Ps<0.001]. Analysis of food lever responding yielded a significant effect of Genotype [$F(4,52)=9.1$, $P<0.01$] and Ethanol concentration [$F(4,52)=4.2$, $P<0.03$] but not Genotype×Ethanol concentration indicating KO mice responded less overall on the food lever during phase 2 compared to WT mice. Analysis of water intake yielded significant effects of Ethanol concentration [$F(4,52)=21.5$, $P<0.001$] and Genotype×Ethanol concentration [$F(4,52)=6.3$,

Table 1 Mean (±SEM) ml total fluid consumed, number of ethanol bouts, number of water bouts, and number of food bouts during phase 1

Genotype	Session					
	1–5	6–10	11–15	16–20	21–25	26–30
Total fluid						
KO	4.7(0.3)	4.2(0.4)	4.3(0.4)	4.2(0.4)	3.8(0.3)	3.5(0.3)
WT	6.3(0.4)	5.1(0.4)	5.0(0.4)	4.6(0.5)	4.6(0.4)	4.5(0.3)
Ethanol bouts						
KO	–	–	–	–	–	–
WT	3(1)	3(1)	4(1)	5(2)	7(1)	7(2)
Water bouts						
KO	32(2)	32(2)	31(2)	32(3)	29(2)	29(2)
WT	41(6)	35(6)	32(6)	32(6)	32(4)	30(3)
Food bouts						
KO	45(2)	42(1)	40(1)	46(4)	42(3)	41(2)
WT	54(2)	45(2)	44(2)	45(2)	44(2)	42(2)

Table 2 Mean (±SEM) ml total fluid consumed, number of ethanol bouts, number of water bouts, and number of food bouts during phase 2

Genotype	Ethanol (% v/v)				
	0	5	10	20	30
Total fluid					
KO	3.8(0.3)	3.6(0.2)	4.2(0.3)	3.9(0.3)	3.8(0.2)
WT	4.6(0.3)	4.5(0.3)	4.7(0.3)	4.4(0.3)	4.7(0.3)
Ethanol bouts					
KO	–	–	–	–	–
WT	5(1)	8(1)	9(1)	7(1)	5(1)
Water bouts					
KO	28(2)	29(2)	32(1)	30(2)	30(2)
WT	32(3)	32(2)	30(3)	33(3)	34(3)
Food bouts					
KO	37(1)	41(2)	43(2)	41(2)	41(2)
WT	42(2)	43(3)	44(2)	42(2)	43(2)

$P<0.001$]. Follow-up analyses within each genotype yielded significant effects of Ethanol concentration [KO: $F(4,28)=8.8$, $P<0.002$; WT: $F(4,24)=16.2$, $P<0.001$]. A significant Genotype effect was seen only at the 20% v/v ethanol concentration [$F(1,13)=9.0$, $P<0.01$].

Analysis of total fluid intake revealed significant effects of Ethanol concentration [$F(4,52)=6.3$, $P<0.001$] and Genotype×Ethanol concentration [$F(4,52)=3.4$, $P<0.03$] (Table 2). Analyses at each ethanol concentration yielded significant effects of Genotype at the 5% ethanol concentration [$F(1,13)=5.3$, $P<0.04$] and the 30% ethanol concentration [$F(1,13)=7.6$, $P<0.02$]. As in phase 1, WT mice responded for ethanol in bouts and KO mice did not. Furthermore, in WT mice, the frequency of ethanol bouts depended on ethanol concentration [$F(4,24)=6.8$, $P<0.005$]. Analysis of water bout frequency yielded a significant Genotype×Ethanol concentration interaction [$F(4,52)=2.9$, $P<0.05$]. However, follow-up comparisons did not indicate significant effects of Ethanol concentration in either genotype or significant Genotype effects at each ethanol concentration. Analysis of food bout frequency yielded a significant effect of Ethanol concentration [$F(4,52)=4.1$, $P<0.02$], but not Genotype or Genotype×Ethanol concentration.

Table 3 Mean (±SEM) ml total fluid consumed, number of ethanol bouts, number of water bouts, and number of food bouts during phase 3

Genotype	Ethanol (% v/v)			
	0	5	10	20
Total fluid				
KO	3.7(0.3)	3.7(0.4)	4.0(0.4)	4.1(0.4)
WT	6.0(0.3)	5.9(0.4)	5.5(0.4)	5.6(0.5)
Ethanol bouts				
KO	–	–	–	–
WT	22(2)	23(3)	25(5)	23(5)
Water bouts				
KO	29(2)	27(2)	28(3)	28(3)
WT	14(4)	11(3)	12(3)	16(3)
Food bouts				
KO	41(2)	43(3)	44(3)	43(3)
WT	43(2)	41(3)	43(3)	44(2)

During phase 3, with saccharin present, WT mice continued to show higher total responses on the ethanol lever than KO mice, with and without ethanol present (Fig. 3). Overall analysis yielded a significant effect of Genotype [$F(1,13)=59.9$, $P<0.001$], but not Ethanol concentration or Genotype×Ethanol concentration. WT mice responded more on the food lever than KO mice with the analysis yielding a significant effect of Genotype [$F(1,13)=6.7$, $P<0.02$], but not Ethanol concentration or Genotype×Ethanol concentration. However, KO mice consumed greater amounts of water than WT mice, with the analysis yielding a significant effect of Genotype [$F(1,13)=29.5$, $P<0.001$].

Analysis of total fluid intake yielded a significant effect of Genotype [$F(1,13)=12.6$, $P<0.004$], but not Ethanol concentration or Genotype×Ethanol concentration (Table 3). WT mice responded on the ethanol lever in bouts, although bout frequency was the same across ethanol concentrations. KO mice generated higher numbers of water bouts compared to WT mice, with the analysis yielding a significant effect of Genotype [$F(1,13)=11.6$, $P<0.005$] but not Ethanol concentration or Genotype× Ethanol concentration. Both genotypes generated the same frequency of food bouts.

Table 4 gives mean (±SEM) g/kg ethanol intakes based on the number of dippers presented during a session. WT mice had higher g/kg ethanol doses/session than KO mice during each phase. Analysis of phase 1

Table 4 Mean (±SEM) g/kg ethanol presented per session, during each experimental phase

Experimental phase	Mean g/kg ethanol/session	
	KO	WT
Phase 1		
Trials		
1–5	1.3(0.4)	2.8(0.4)
6–10	1.1(0.2)	2.6(0.3)
11–15	0.8(0.3)	3.0(0.3)
16–20	0.7(0.4)	3.5(0.4)
21–25	0.6(0.4)	3.8(0.4)
26–30	0.5(0.4)	4.3(0.5)
Phase 2		
Ethanol (% v/v)		
5	0.2(0.1)	1.5(0.2)
10	0.3(0.3)	3.2(0.3)
20	0.8(0.9)	9.7(0.9)
30	1.2(1.5)	12.3(1.6)
Phase 3		
Ethanol (% v/v)		
5	0.9(0.5)	6.2(0.5)
10	1.6(1.0)	11.2(1.1)
20	3.3(2.5)	21.0(2.7)

doses yielded a significant effect of Genotype [$F(1,13)=48.9$, $P<0.001$] and Genotype×Trial block [$F(5,65)=5.3$, $P<0.02$]. Significant changes in dose levels over trial block were not seen in either genotype. However, significant Genotype effects were seen at each trial block [all Fs(1,13)>8.6, Ps<0.02]. Analysis of phase 2 doses yielded significant effects of Genotype [$F(1,13)=35.8$, $P<0.001$], Ethanol concentration [$F(3,39)=31.4$, $P<0.001$], and Genotype×Ethanol concentration [$F(3,39)=21.6$, $P<0.001$]. Significant ethanol concentration effects were seen in each genotype [KO: $F(3,21)=13.7$, $P<0.002$; WT: $F(3,18)=23.2$, $P<0.001$], and significant effects of genotype were seen at each ethanol concentration [all Fs(1,13)>23.9, Ps<0.001]. Analysis of phase 3 doses also yielded significant effects of Genotype [$F(1,13)=32.2$, $P<0.001$], Ethanol concentration [$F(2,26)=27.9$, $P<0.001$], and Genotype×Ethanol concentration [$F(2,26)=14.4$, $P<0.002$]. Significant effects of ethanol concentration were seen in each genotype [KO: $F(2,14)=14.7$, $P<0.003$; WT: $F(2,12)=18.7$, $P<0.004$], and significant effects of genotype were seen at each ethanol concentration [all Fs(1,13)>22.7, Ps<0.001].

Table 5 shows mean (±SEM) ethanol bout size in each phase for WT mice. Repeated measures analyses showed the numbers of dippers per bout remained constant throughout each phase. Table 5 also shows the proportion of prandial and non-prandial ethanol bouts for each phase. Both types of bouts were noted in each phase, of which non-prandial bouts constituted approximately 30–50% of the total number.

KO mice tended to weigh less than WT mice throughout the experiment. Mean (±SEM) weight (g) for each genotype are as follows: phase 1; KO, 26.4±0.7; WT, 28.8±0.8; phase 2; KO, 27.5±0.8; WT, 29.9±0.8; phase 3; KO, 28.0±0.8; WT, 30.5±0.9. Analyses yielded a significant effect of genotype during phase 1 [$F(1,13)=4.7$, $P<0.05$] but not in phase 2 or phase 3.

Table 5 Mean (±SEM) number of dippers per ethanol bout, number of prandial ethanol bouts, non-prandial ethanol bouts and percentage of total bouts that were non-prandial in WT mice during each experimental phase

Experimental phase	Bout size/session	Prandial bouts	Non-prandial bouts	% Non-prandial
Phase 1				
Trials				
1–5	5.6(0.4)	2(1)	2(0)	50
6–10	5.9(0.7)	2(1)	1(1)	33
11–15	5.5(0.2)	3(1)	2(1)	40
16–20	5.6(0.4)	3(1)	2(1)	40
21–25	5.9(0.4)	4(1)	2(1)	33
26–30	6.0(0.4)	4(1)	3(1)	43
Phase 2				
Ethanol (% v/v)				
0	6.8(0.8)	4(1)	2(0)	33
5	6.0(0.4)	3(1)	2(1)	40
10	7.3(0.9)	5(1)	3(1)	38
20	6.8(0.7)	7(1)	3(1)	30
30	6.8(0.6)	5(1)	2(1)	29
Phase 3				
Ethanol (% v/v)				
0	7.1(0.8)	12(1)	10(1)	45
5	7.1(0.7)	12(2)	11(2)	48
10	7.1(0.8)	11(2)	9(2)	45
20	7.5(1.1)	11(2)	8(2)	42

Discussion

The present study examined operant ethanol self-administration in D_2 KO mice using a relatively long-term procedure with results demonstrating that disabling of D_2 receptors produces a profound reduction in ethanol-seeking behavior. KO mice consistently responded less on the ethanol-associated lever across a variety of ethanol-concentration conditions compared to WT mice. These results compare favorably with the results obtained using a home cage two-bottle drinking procedure where KO mice drank less ethanol than WT mice (cf. Phillips et al. 1998). However, KO mice also responded less for food in several conditions. In addition, KO mice responded less on the fluid lever in the absence of ethanol when either plain water or 0.2% saccharin was available. Thus, overall the KO mice were less vigorous responders, indicating a non-specific decrement in reinforcer-seeking behavior.

In a similar procedure, C57 mice display patterns of responding similar to that seen with the WT mice used in the present study (cf. Risinger et al. 1998). C57 mice respond at high levels for access to ethanol compared to

DBA mice. C57 mice show ethanol concentration-dependent responding, indicating ethanol is an effective reinforcer in this strain. C57 mice also show increased lever responding when saccharin (with or without ethanol) is available, and generate an episodic bout pattern of responding. The behavior of the WT mice in the present study is consistent with their C57 background. That is, they showed relatively high levels of responding for ethanol in each phase. Response rates for dipper access were dependent on ethanol concentration in phase 2, and they responded at higher levels when saccharin was available. WT mice generated a bout pattern of responding similar to that seen in C57 mice. Notably, bout size remained relatively constant, with changes in ethanol intake determined by the number of bouts rather than bout size, which is similar to the behavior of C57 mice (Risinger et al. 1998) and ethanol preferring P rats (Files et al. 1993).

D_2 receptor function has been implicated in ethanol drinking and ethanol reinforced behavior in rats by the use of pharmacological agents (e.g., D_2 antagonists) with at least some studies showing reductions in drinking or responding for ethanol (e.g., Pfeffer and Samson 1986, 1988). As previously indicated, based on data from a study with the same mouse genotypes as used in the present study, D_2 receptor function appears particularly important for ethanol drinking in mice (Phillips et al. 1998). Ethanol's effect on activity is also blunted in the D_2 KO mice, confirming results seen with the D_2 antagonist haloperidol (Risinger et al. 1992). In general, the present pattern of results confirms the role of D_2 receptors in ethanol drinking. That is, D_2 KO mice consistently responded less for access to ethanol compared to WT mice.

Reduced sensitivity to ethanol's rewarding effects is one interpretation of the low ethanol preference seen in D_2 mice (Phillips et al. 1998), and the lack of operant ethanol self-administration noted in the present study. However, KO mice also responded less for water, food and saccharin. The lower responding of KO mice may, in part, reflect the locomotor decrement noted in this genotype (Kelly et al. 1998). In addition, the reduced levels of responding for several reinforcers is suggestive of a non-specific role of the D_2 receptor in mediating reinforcement-related behaviors. For example, D_2 receptor function has been suggested as necessary for the experience of reward (Wise et al. 1978). Alternatively, dopaminergic function may regulate the production of instrumental responding rather than the direct effects of rewarding stimuli (Salamone et al. 1997). For example, reduced dopamine levels are associated with impaired operant responding for food only at higher FR ratios (Aberman and Salamone 1999). In accord with this view, D_2 KO mice have a similar preference for saccharin (Phillips et al. 1998), but appear less willing to perform the lever press requirement in order to gain access to this substance compared to the WT mice. However, in the present study, saccharin was presented after a long number of sessions with only ethanol and water available, thus the failure to respond for saccharin may reflect the previous association of the lever response with the less preferred solutions. It may be noted, however, that DBA mice increased responding when saccharin was presented in a similar procedure with a large number of pre-saccharin sessions (Risinger et al. 1998). The suggestion of a non-specific effect of D_2 receptors on motivated behaviors is also supported by the finding of reduced responded in the KO mice during training sessions. The present training and initiation sequence likely contributed to genotype differences seen in subsequent ethanol responding during the continuous access phases. For example, an extended ethanol initiation sequence might have enhanced ethanol responding in KO mice. Also, prior exposure to sucrose during the training sequence could have influenced ethanol self-administration levels. Reduced response levels and reinforcer presentations during training and initiation may have prevented the KO mice from learning the relationship between lever responding and the pharmacological effects of ethanol and influenced later responding during the continuous access phases. However, KO mice were able to acquire the lever response, and received access to meaningful amounts of ethanol during these sessions, although these levels were below that seen in WT mice. Thus, D_2 mice may in fact be less sensitive to a variety of reinforcers when presented with a greater work requirement (e.g., lever press) than used in home cage drinking procedures.

In summary, the present results are consistent with the involvement of dopaminergic systems in ethanol drinking or reinforcement, and are also consistent with a general involvement in instrumental behaviors. Although operant procedures and two-bottle choice drinking procedures yield similar patterns of genetic differences in some cases (e.g., C57 mice drink and respond more compared to DBA mice, cf. Belknap et al. 1993; Risinger et al. 1998), the present operant procedure has also failed to identify differences in operant responding in genotypes showing differences in ethanol drinking. Specifically, serotonin 5-HT_{1b} receptor KO mice drink large amounts of ethanol, yet did not respond for ethanol in the operant setting (Risinger et al. 1999b). Although D_2 receptors appear important for both ethanol drinking and reinforcement, it remains unclear whether the hedonic mechanism is a reduction in ethanol reward. As previously reviewed, pharmacological treatments aimed at reducing D_2 receptor function produced both decreases and increases in ethanol consumption (e.g., Hubbell et al., 1991; Dyr et al. 1993). Further, ethanol preferring rats show lower D_2 receptor binding (Stefanini et al. 1992; McBride et al. 1993), which would suggest KO mice would consume greater amounts of ethanol than WT mice. However, ethanol preferring C57 mice have higher D_2 binding compared to ethanol avoiding DBA mice (Ng et al. 1994), which corresponds to the reduction in ethanol preference seen in KO mice. Finally, the D_2 antagonist haloperidol is ineffective in reducing ethanol reward measured in the place conditioning paradigm (Risinger

et al. 1992), but reduces ethanol conditioned taste aversion (Risinger et al. 1999a). Thus, the role of D_2 receptors in ethanol's motivational appears complex and likely depends on the actions of several other neurotransmitter systems (Koob et al. 1998).

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