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ORIGINAL INVESTIGATION

Behavioral economics of food reinforcement and the effects of prefeeding, extinction, and eticlopride in dopamine D_2 receptor mutant mice

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Abstract
Rationale Several studies have investigated the reinforcing effects of food in genetically engineered mice lacking dopamine D_2 receptors (DA D_2Rs); however, behavioral economic analyses quantifying reinforcement have not been conducted.
Objective The role of DA D_2Rs in food reinforcement was examined by comparing responding under various fixed-ratio (FR) schedules of reinforcement, and effects of extinction, satiation, and the DA D_2R antagonist eticlopride, in mice with and without genetic deletions of the receptor.
Results Response rates of DA D_2R knockout (KO) mice were generally lower than those of littermate wild-type (WT) and heterozygous (HET) mice. The demand curve (consumption vs. FR value) for KO mice decreased more steeply than that of HET or WT mice, suggesting that reinforcing effectiveness is decreased with DA D_2R deletion. Prefeeding decreased, whereas extinction increased overall response rates as a proportion of baseline, with no significant genotype differences. Both (+)- and (−)-eticlopride dose-dependently decreased responding in all genotypes with (−)-eticlopride more potent than (+)-eticlopride in all but KO mice. The enantiomers were equipotent in KO mice, and similar in potency to (+)-eticlopride in WT and HET mice.
Conclusions That prefeeding and extinction did not vary across genotypes indicates a lack of involvement of DA D_2Rs in these processes. Differences between (−)-eticlopride effects and extinction indicate that DA D_2R blockade does not mimic extinction. The maintenance of responding in KO mice indicates that the DA D_2R is not necessary for reinforcement. However, the economic analysis indicates that the DA D_2R contributes substantially to the effectiveness of food reinforcement.

Keywords Behavioral economics · Dopamine D_2 receptors · Knockout mice · Eticlopride · Fixed-ratio schedule of reinforcement

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Introduction

The importance of dopamine (DA) in mediating the reinforcing effects of a wide variety of stimuli is suggested by findings including those demonstrating that drugs that directly or indirectly stimulate DA receptors are self-administered (Deneau et al. 1969; Goeders and Smith 1983; Wise et al. 1976) and the findings that natural reinforcers such as food and water produce increases in extracellular DA (Hajnal et al. 2004; Hernandez and Hoebel 1988; Yoshida et al. 1992). Further, it has been suggested that DA antagonists produce within- and across-session changes in responding maintained by food reinforcement that resemble the changes in responding that occur when reinforcement is discontinued (extinction). For example, Wise et al. (1978) found that

moderate doses (0.5 and 1.0 mg/kg) of pimozide produced within-session decreases in response rates and repeated administration of pimozide produced across-session declines in responding, results similar to what is seen when reinforcement is discontinued. This finding has been interpreted as a DA antagonist produced decrease in reinforcing effectiveness. Alternatively, some researchers have argued that the effects of DA receptor antagonism depend on the number of responses required for reinforcement and should not be interpreted as a general decrease in reinforcing effectiveness (Salamone and Correa 2002; Salamone et al. 1997).

A behavioral economic approach (Hursh 1980, 1984; Hursh et al. 1988) to the assessment of reinforcing effectiveness is based on assessing how reinforcer consumption changes as a function of changes in price (the number of responses required per reinforcer). Specifically, the primary measure of interest is the rate of decline in consumption with increases in price. When demand decreases rapidly with increasing price, a reinforcer is considered less effective and demand more "elastic." When demand decreases slowly (or not at all) with increasing price, a reinforcer is considered more effective and demand "inelastic." Hursh and Silberberg (2008) have provided an account (exponential model of demand) for scaling the rate of decline in consumption with a single parameter (α), which measures the effectiveness of a reinforcer.

The development of genetically modified mice has allowed the investigation of the role of particular receptors in mediating behavior. Because DA is thought to be involved in mediating reinforcing effects, genetically modified mice may provide insight into the role of DA receptors. Several studies have used mutant mice to investigate the role of DA D_2 receptors (DA D_2Rs) in the reinforcing effects of a variety of drugs with some studies concluding a role for DA D_2Rs in reinforcing effects (Elmer et al. 2002; Risinger et al. 2000) and another not (Caine et al. 2002).

The present study used a variety of methods to assess the role of DA D_2Rs in mediating the reinforcing effectiveness of food in DA D_2 knockout (KO), wild-type (WT), and heterozygote (HET) mice. In the first set of experiments, the ratio required for food delivery was increased from 5 to 160 and the changes in consumption as price increased were used to compare the reinforcing effectiveness of food across the three genotypes of mice. Second, the responses of these genetic lines of mice to food satiation or extinction, processes that are involved in food reinforcement, were also assessed. Finally, differences between genotypes in the effects of the D_2R antagonist (−)-eticlopride and its (+)-enantiomer were determined, and those effects in WT mice were compared to the effects of extinction to determine whether blockade of D_2Rs appears equivalent to extinction. Because results can depend on response type (Caine et al. 1999; Goeders et al. 2009), the generality of results was assessed by examining effects with both lever-pressing and nose-poking responses.

Methods

Subjects DA D_2R WT (n=13), HET (n=12), and KO (n=12) mice were singly housed under a 12-h/12-h light/dark cycle (lights on at 0700 hours). The mice averaged 24 weeks of age when experiments started. The (incipient) congenic mice used in the present studies were the product of at least ten generations of mating HET mice with WT C57BL/6 J mice. The homologous recombination techniques, specific details of the DA D_2R targeting methodology, and characterization of these mutant mice have been reported elsewhere (Kelly et al. 1997, 1998).

The mice were maintained at 85% of their unrestricted feeding weights for the duration of the study. All testing was performed between 1300 and 1600 hours. Mice were fed daily rations at least 1 h after behavioral testing. The animals were maintained in an AAALAC International accredited facility in accordance with NIH Policy Manual 3040-2, Animal Care and Use in the Intramural Program (released 1 November 1999).

Drugs The (+)- and (−)-enantiomers of eticlopride HCl (Research Biochemicals, Inc.) were dissolved in a 0.9% saline vehicle and injected i.p. in a volume of 10 ml/kg. Injections were administered, the mouse was place in the experimental chamber, and the session started 5 min later.

Apparatus Experimental sessions were conducted in operant conditioning chambers (Med Associates, modified Model ENV 307A, St. Albans, VT) measuring 15.9 (length)×14 (depth)×12.7 (height) cm. The front wall of each chamber contained either two levers (each requiring a force of approximately 2 g to depress) or two nose poke holes equipped with infrared detection (each requiring entry of the nose to approximately 0.64 cm to break the beam) located equidistant from the center of the wall. Mounted behind the front wall was a dispenser for the delivery of food pellets (20-mg Precision food pellet, BioServ, Frenchtown, NJ) into a food cup located in the center of the front wall between the two response levers or the nose poke holes. Centered above each lever or nose poke hole was a row of three light-emitting diodes, and a single 28-V d.c. lamp (houselight) that provided general illumination was mounted in the center at the top of the front wall.

Procedure Experimental sessions were conducted once per day, 5 days per week. Sessions began 5 min after the subject was placed in the chamber with illumination of the houselight and the stimulus lights above the levers or nose poke holes. One group of mice of each genotype (seven WT, six HET, and six KO) was initially trained to press either lever and a second group of mice (six WT, six HET, and six KO) was trained to poke their nose in either hole,

with each response producing a food pellet. Once responding occurred reliably under fixed-ratio (FR) 1, a 10-s timeout following pellet delivery was introduced. During the timeout, all lights were off and responses were recorded, but had no scheduled consequences. Subsequently, the lights over the left lever or left nose poke hole were turned off, only responses on the right lever or right nose pokes produced reinforcers, and the FR value remained at 1. Sessions ended after completion of 20 ratios or 30 min, whichever occurred first.

After two sessions in which 20 ratios were completed, the FR value was first increased to 5 for twenty sessions and subsequently doubled every twenty sessions until it reached 160. Next, the FR value was reduced to 5 and the effects of prefeeding were determined. Mice were fed either twice or four times their daily feed amounts following selected sessions and responding during the following day's session was compared to the immediately preceding (baseline) sessions. During another selected session, extinction was in effect. Extinction was implemented by diverting the path of delivered pellets to a cup located outside the chamber. All other aspects of these sessions (e.g., stimulus changes) were similar to those conducted with the FR 5-response schedule in effect. A minimum of 7 and an average of 23 days occurred between each prefeeding session to eliminate any effects of prefeeding that persisted beyond the designated session.

Finally, with the FR retained at 5, the effects of the DA D_2R antagonist eticlopride were determined. Five minutes before some sessions mice were injected with vehicle (saline), (−)-eticlopride (0.1–30 mg/kg), or (+)-eticlopride (3–30 mg/kg). Doses were selected on the basis of previous studies (Chausmer and Katz 2001) and tested in ascending order. At least two baseline (no injection) sessions occurred between eticlopride test sessions.

Data analysis Overall response rate was calculated for each subject as the number of responses divided by total time in the session excluding timeouts. The post-reinforcement pause (PRP) was calculated by summing the time to the first response in each FR (from the end of the timeout or the start of the session) and dividing by the number of pauses obtained. Response rates were also calculated excluding PRP (run rate; total responses divided by total session time excluding timeouts and PRP values). Individual subject averages for each measure were calculated over the last three sessions at each FR value and used to calculate averages for each genotype. For effects of prefeeding, extinction, or drug tests, response rates were calculated across all sessions that preceded tests (the average from the three sessions that preceded the two prefeeding and the one extinction session were used as the baseline for those sessions; the average from all vehicle sessions that preceded each eticlopride test session were used as the baseline for all eticlopride sessions), and those averages were then used as baseline to calculate percentages of control values. Separate two-way repeated measures ANOVAs, one for each response type, were conducted to assess the effects of these treatments. Selected post-hoc comparisons were conducted using the Holm–Sidak method. For the effects of eticlopride, linear regression was used to calculate ED_{50} values (dose producing a 50% reduction in responding relative to control sessions) using the linear portions of the dose–effect curves (Snedecor and Cochran 1967). Pairs of ED_{50} values were considered significantly different when their 95% confidence limits did not overlap. Parallel-line bioassay was used to assess potency of (+)- relative to (−)-eticlopride (Finney 1964). The value obtained is the dose of (+)-eticlopride equal to 1 mg/kg of (−)-eticlopride. A significant relative-potency difference is indicated when the 95% confidence limits for that value do not include 1.0.

Based on Hursh and Silberberg (2008), the logs of the group average of reinforcers obtained vs. FR value were fitted with the equation $\log Q = \log Q_0 + k(e^{-\alpha Q_0 C} - 1)$ separately for each genotype to provide estimates of maximum consumption at zero price, Q_0, and α, the rate of decline, using Graphpad Prism version 5 (Graphpad Software, Inc., San Diego, CA.). According to the equation, C represents the cost of each reinforcer (i.e., FR value) and k is the span of the function and was set to 4 for all data sets. To determine if the α values obtained for each genotype were statistically different, the separate fits were compared via an F test to a global fit obtained utilizing a common α value for the three genotypes (Motulsky and Christopoulos 2004).

Results

The unrestricted feeding weights before deprivation and training of the three genotypes were not significantly different ($F_{2,34}$=0.74, p=0.48; averages of 24.9, 26.8, and 26.3 g for the DA D_2R WT, HET, and KO mice, respectively). For lever pressing, overall response rates (Fig. 1, top panels) of WT mice were higher than those of HET and KO mice ($F_{2,15}$=18.8, p<.001; Holm–Sidak post hoc comparisons), whereas for nose poking, overall response rates of the WT mice were only higher than those of the KO mice ($F_{2,15}$=12.04, p<.001). Run rates (Fig. 1, middle panels) for lever pressing were higher in WT than HET or KO mice ($F_{2,15}$=14.952, p<0.001; Holm–Sidak post hoc comparisons) whereas run rates for nose poking were higher in WT compared to KO mice, but not in WT compared to HET mice ($F_{2,15}$=5.781, p=.013; Holm–Sidak post hoc comparisons). Finally, PRP values (Fig. 1, bottom panels) were lower in WT mice compared to KO mice for

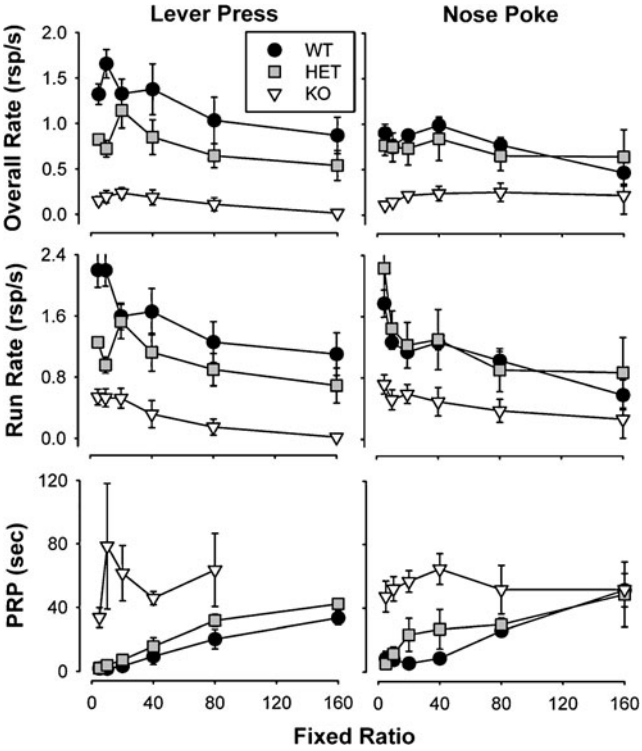

Fig. 1 Changes in overall response rate (*top panels*), run rate (*middle panels*), and post-reinforcement pause (*bottom panels*) as a function of FR value for lever pressing (*left column of graphs*) or nose poking (*right column of graphs*). Each data point represents the average across individual mice within a genotype. *Error bars* represent ±standard error of the mean. Note that no PRP values are plotted for the KO mice for lever pressing under FR 160 because no reinforcers were obtained at that FR value

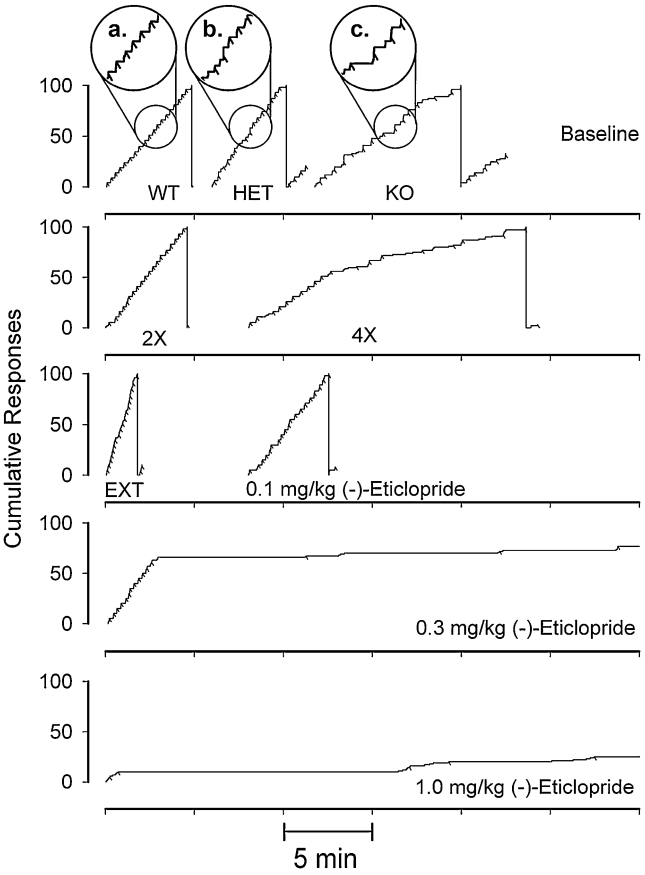

Fig. 2 Representative nose poking cumulative records of performances from a DA D_2R WT, HET, and KO mouse. *Ordinates* cumulative responses. *Abscissae* time (with calibrations in 5 min increments). *Diagonal marks* indicate reinforcer deliveries except during extinction where they indicate operation of the feeder and associated stimulus changes. The recording runs continuously throughout experimental sessions, including the 10-s timeout periods following pellet delivery. The *top row* shows baseline records of performances. Note that records for HET and KO mice are displaced to the *right*. The *encircled sections* are magnified 200-fold. The second row shows records from the same WT mouse as in the first row during prefeeding of either twice (2×) or four times (4×) its daily amount, on the day prior to the selected session. The *third row* shows the effects of extinction and a low dose of (−)-eticlopride, with higher doses displayed in the *fourth* and *fifth rows*, again for the same WT mouse as in the *first row*

nose poking ($F_{2,15}$=12.369, p<.001). A failure to obtain reinforcers at the highest FR in some mice precluded a statistical analysis of PRP values for lever pressing.

For the D_2R WT and HET mice under the FR 5 schedule, a typical FR pattern of responding was obtained wherein reinforcer delivery was followed by a short pause and then by rapid completion of the required ratio of responses (see top row in Fig. 2, records above "WT" and "HET" and insets a and b). For the D_2R KO mice, the overall rate was lower, consistent with the results in Fig. 1; however, response rates were on occasion as high as those seen with WT mice. In addition, the pattern of responding in the DA D_2R KO mice differed from that of the WT mice, with responses sometimes occurring just after reinforcer delivery and pauses occurring within ratios (see top row in Fig. 2, record above "KO" and inset c).

For the three genotypes and both response types, number of reinforcements decreased with increases in FR schedule requirement; in behavioral economic terms, consumption decreased as a function of price. As is characteristic of similar studies, the size of the decreases in consumption increased with increases in price (Fig. 3). Equation 1 (lines in Fig. 3) described the data for both lever pressing and nose poking and for all three genotypes, with the fits accounting for 89% to 99% of the variance (Table 1). The obtained α values, the parameter representing the inverse of reinforcer effectiveness, were largest for the DA D_2R KO mice, smallest for the WT mice, and intermediate for the HET mice. One-way ANOVAs indicated significant effects of genotype on the obtained α values for lever pressing ($F_{2,11}$=122.1, p<0.0001) and nose poking ($F_{2,12}$=34.1, p<0.0001). Follow-up ANOVAs using a corrected criterion level (α=0.025) revealed that values for the parameter α obtained for the WT fits were significantly greater than the

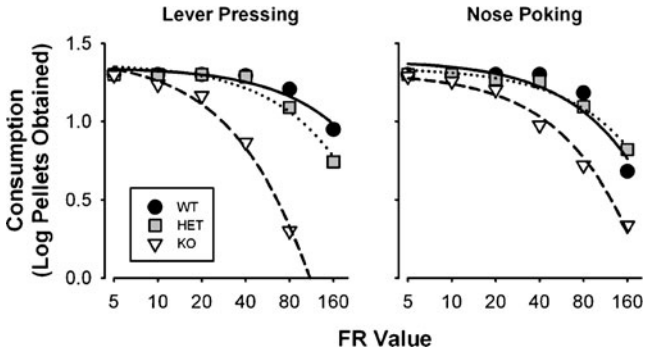

Fig. 3 Changes in consumption as a function of price (FR value). Consumption decreased as a function of increases in price for all three genotypes and for both lever pressing (*left panel*) and nose poking (*right panel*). Consumption decreased more rapidly for KO than HET or WT mice for both lever pressing and nose poking (i.e., consumption of KO mice was more elastic). Each *data point* is the average across individual mice. Changes in consumption as a function of price were well described by the exponential model of demand (*solid line*, *dotted line*, and *dashed lines* are best fitting curves for WT, HET, and KO mice, respectively) and obtained estimates of α were consistent with the suggestion that reinforcer effectiveness was lowest for KO mice, intermediate for HET mice, and highest for WT mice (see Table 1)

α values obtained for the KO fits ($F_{1,7}$=277.4, p<0.0001 and $F_{1,8}$=35.1, p=0.0004 for lever pressing and nose poking, respectively). Follow-up ANOVAs also revealed that the obtained α value for the WT fit was significantly greater than the α value for the HET fit for lever pressing ($F_{1,8}$=12.5, p=0.0077), but not for nose poking.

Prefeeding decreased overall response rates in a manner that depended on the amount of food delivered the day before (Fig. 4 top row, bars above 2× and 4×). Prefeeding four times the normal amount increased the length of PRP values (Fig. 4 and in Fig. 2, compare performance of WT in the first row to that in the second row). Similar effects were obtained with each genotype and response type. ANOVA confirmed an effect of prefeeding amount on overall response rate for both response types ($F_{2,30}$=88.8, p<.001 and $F_{2,28}$=41.1, p<.001 for lever pressing and nose poking, respectively). Decreases in overall response rate due to prefeeding were attributable to significant decreases in run rate (lever press, $F_{2,30}$=140, p<.001; nose poke, $F_{2,28}$= 16.2, p<.001) and increases in PRP values (lever press, $F_{2,30}$=20.9, p<.001; nose poke, $F_{2,28}$=11.5, p<.001). Although the difference was small, overall response rates as a percentage of baseline were higher in WT mice than HET mice for lever pressing ($F_{2,15}$=3.98, p=.04; post hoc comparison), but not for nose poking. There were no significant effects of genotype on run rate or PRP for either response type.

Extinction produced increases in overall response rate for all three genotypes and for both response types (Fig. 4, top row, bars above EXT). The cumulative records of responding show that extinction produced a decrease in PRP values that contributed to the increase in overall response rate (Fig. 2, compare top and third rows). The increases in overall response rate produced by extinction were statistically significant for both response types (lever press, $F_{1,14}$=12.2, p=.004; nose poke, $F_{1,14}$=19.4, p<.001), whereas no statistically significant effect of genotype on overall response rate was obtained. The increases in overall response rate under extinction were due to decreases in PRP (lever press, $F_{1,14}$=532, p<.001; nose poke, $F_{1,14}$=727, p<.001) and not increases in run rate (lever press, $F_{1,14}$=0.772, p=.394; nose poke, $F_{1,14}$=.238, p=.633). No significant effect of genotype was obtained for run rates or PRP values.

In DA D_2R WT mice, both enantiomers of eticlopride decreased rates of lever pressing and nose poking (Fig. 5, top row). Of the two enantiomers, (−)-eticlopride was 36-fold (lever press) or 23-fold (nose poke) more potent than (+)-eticlopride at reducing rates of responding in the WT mice, as judged by significant relative potency estimates (Table 2). In the HET mice, (−)-eticlopride also decreased rates of either response with about 38-fold greater potency than (+)-eticlopride. Both enantiomers of eticlopride also decreased rates of either response in the DA D_2R KO mice. Decreases in response rates in the KO mice, however, were only obtained at doses of either enantiomer greater than 10 mg/kg for both response types (Fig. 5, bottom row). There were no differences in the potency of (−)- and (+)-eticlopride in the DA D_2R KO mice for either response, as indicated by the nonsignificant relative potencies obtained (Table 2). In addition, the potency of (−)-eticlopride in the KO mice was less than that in the WT or HET mice (Table 2, Fig. 5).

A low dose of (−)-eticlopride did not alter the pattern of responding (Fig. 2, compare top and third rows). At higher doses of (−)-eticlopride, responding appeared normal at the beginning of the session followed by an abrupt change to

Table 1 Estimated parameters, Q_0 and α, and percentage of variance accounted for (%VAF) from fits of the exponential model of demand

Parameters	Lever Pressing			Nose Poking		
	WT	HET	KO	WT	HET	KO
α	2.69×10^{-5}	4.30×10^{-5}	1.52×10^{-4}	4.36×10^{-5}	3.85×10^{-5}	8.91×10^{-5}
Q_0	22.4	23.5	25.9	24.5	22.1	20.5
%VAF	93.3	95.8	99.0	89.0	98.2	98.8

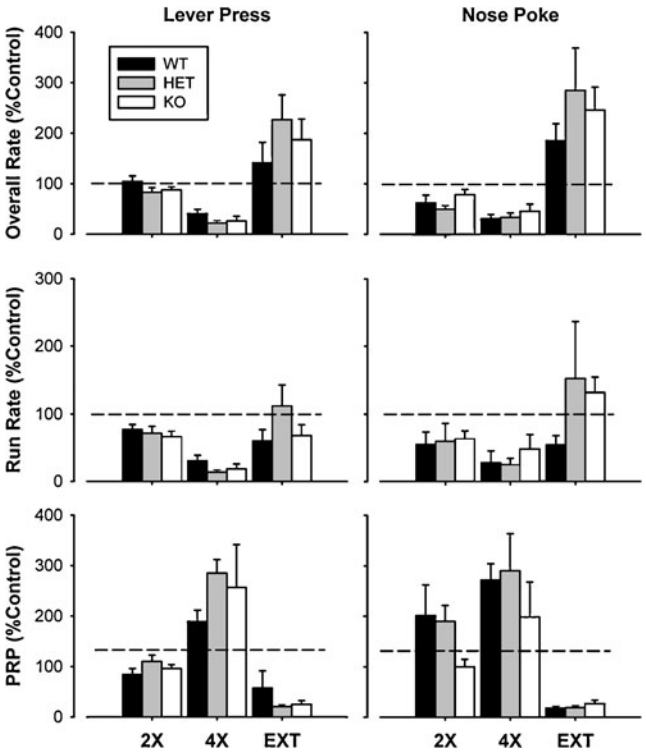

Fig. 4 Changes in overall response rate (*top row*), run rate (*middle row*), and PRP value (*bottom row*), expressed as a proportion of baseline responding for lever pressing (*left column*) and nose poking (*right column*), during sessions following days when mice were fed either double (2×) or quadruple (4×) their daily feed amounts or in which responding no longer resulted in pellet delivery (*EXT*). *Each bar* represents the average across individual mice. *Error bars* represent ±standard error of the mean

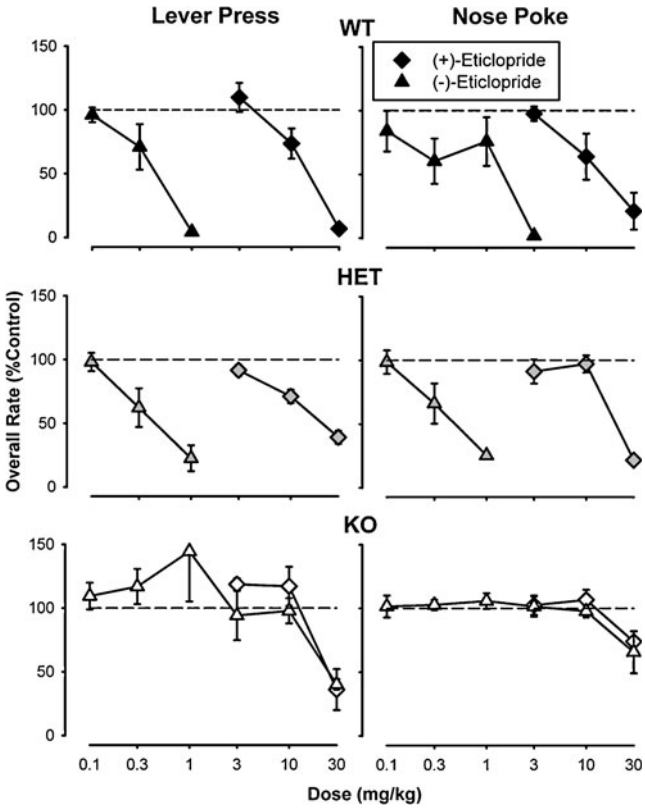

Fig. 5 Changes in overall response rate (*top row* WT, *middle row* HET, *bottom row* KO), expressed relative to baseline response rates for lever pressing (*left column*) and nose poking (*right column*), during sessions preceded by injection (i.p.) of either (+)- or (−)-eticlopride. *Each symbol* represents the average across individual mice. *Error bars* represent ±standard error of the mean

virtually no responding (Fig. 2, compare top and fourth rows). The number of ratios completed before the transition decreased as dose of (−)-eticlopride increased (Fig. 2, compare fourth and fifth rows).

The effects of (−)-eticlopride (the more potent enantiomer) and extinction in WT mice were compared to determine whether the effects of (−)-eticlopride could reasonably be interpreted as resulting from a blockade of the reinforcing effects of food. As indicated above, extinction produced increases in overall response rate (see Fig. 4, top row) whereas (−)-eticlopride only produced decreases in overall response rate (see Fig. 5, top row) suggesting differences in their effects. A detailed comparison of effects of (−)-eticlopride and extinction within sessions (Fig. 6) shows that the response rate increase in extinction was evident after the first completed FR for both response types (Fig. 6, top row). In comparison, low doses of (−)-eticlopride produced minimal effects on either response type (Fig. 6, bottom row) whereas higher doses (1.0 mg/kg for lever pressing and 3.0 mg/kg for nose poking) produced substantial decreases in response rates either initially (nose poking) or gradually over the first ten FR completions (lever pressing).

Discussion

The present study evaluated the specific role of DA D_2Rs in mediating reinforcing effects of food using genetic deletion of the DA D_2R. Absolute rates of food-maintained responding in D_2R KO mice were lower than those in HET or WT mice, consistent with previous studies with food and water reinforcement (Caine et al. 2002; Elmer et al. 2002; Risinger et al. 2000). The fact that lever pressing and nose poking was established and maintained by contingent food delivery in D_2R KO mice demonstrates that the DA D_2R was not *necessary* for food to function as a reinforcer. However, the behavioral economic analysis (Hursh and Silberberg 2008) of decreases in consumption with increases in price indicated that food's reinforcing effectiveness was lowest for DA D_2R KO mice, intermediate for HET mice, and highest for WT mice. The results indicate that, while not necessary for food reinforcement, DA D_2Rs *are* substantially involved in the reinforcing effects of food (cf., Epstein et al. 2007; Wang et al. 2001). Thus, the role of the DA D_2R in reinforcement appears to be modulatory rather than essential, consistent with the

Table 2 Linear regression-calculated doses of (+) and (−)-eticlopride that produced 50% reduction in overall response rate and the 95% confidence limits (CLs) around those doses along with the relative potency of (−)-eticlopride vs. (+)-eticlopride

Genotype	Response	(−)-Eticlopride ED_{50} (mg/kg)	(+)-Eticlopride ED_{50} (mg/kg)	Relative Potency
	Lever Press	0.37	13.1	36.1
WT		(0.27–0.55)	(10.0–18.0)	(24.0–55.1)
	Nose Poke	0.76	13.5	23.3
		(0.28–8.69)	(8.00–32.6)	(7.61–77.8)
	Lever Press	0.43	20.6	39.0
HET		(0.29–0.75)	(15.3–31.7)	(23.7–66.4)
	Nose Poke	0.47	19.9	38.5
		(0.30–0.98)	(17.7–22.6)	(23.1–65.7)
	Lever Press	24.8	24.8	0.94
KO		(18.0–55.8)	(17.7–53.4)	(0.47–2.13)
	Nose Poke	N.S.	67.1	1.34
			(34.2->1,000)	(0.60–4.74)

N.S. non-significant linear regression

likelihood that multiple receptor or neurotransmitter systems have overlapping, or even redundant, functionality in a process as evolutionarily important as food reinforcement.

DA involvement in reinforcement has been experimentally assessed in a number of different ways (Salamone and Correa 2002; Salamone et al. 1997). Several studies have used DA receptor antagonists to acutely block the effects of DA and compared the resulting behavioral change to that of removing reinforcement entirely (extinction). In the present study, the effects of enantiomers of the DA D_2R antagonist eticlopride were examined on responding maintained under an FR 5 schedule of reinforcement. Both (+)- and (−)-eticlopride decreased response rates, with the (−)-enantiomer more potent in DA D_2R WT and HET mice. In the KO mice, the two enantiomers were equipotent, and equally potent with (+)-eticlopride in the WT and HET mice. These results

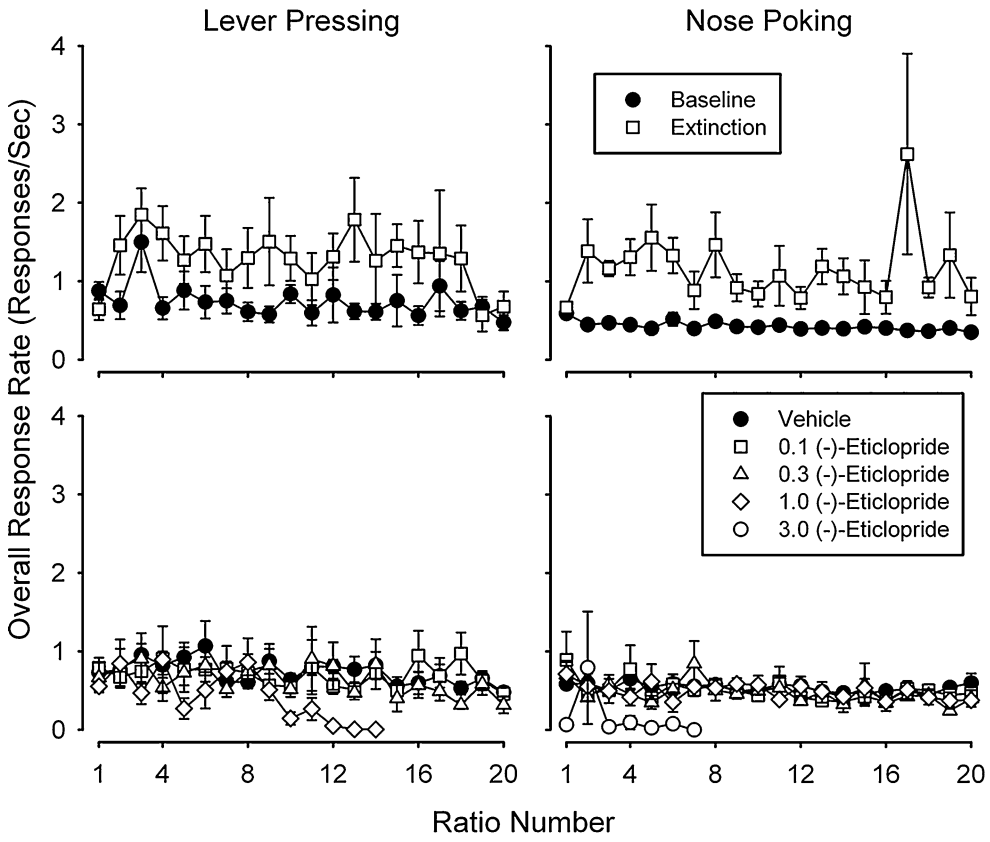

Fig. 6 Response rate plotted as a function of each ratio in the session for WT mice for sessions during which extinction was in effect (*top row*) or sessions following administration of (−)-eticlopride (*bottom row*) for lever pressing (*left column*) and nose poking (*right column*). *Each symbol* represents the average across individual mice. *Error bars* represent ±standard error of the mean

indicate that the decreases in response rates produced by (−)-eticlopride in DA D_2R WT and HET mice were due to DA D_2R antagonism. In addition, the effects of the (+)-enantiomer in all three genotypes were not mediated by DA D_2Rs, as the (+)-entantiomer was equipotent in all three genotypes. Finally, the effects of (+)-eticlopride in the WT and HET mice were not due to enantiomeric impurities, as the effects of the (+)-enantiomer were comparable in mice with and without the receptor at which the (−)-enantiomeric impurity would exert its actions.

Whereas eticlopride decreased response rates in the DA D_2R WT and HET mice, extinction increased response rates. These differences in effects of extinction and DA D_2R antagonist administration contrast with previous suggestions of equivalence between DA antagonism and extinction (Wise 2004, 2008; Wise et al. 1978). One difference between the current study and those indicating equivalent effects of extinction and effects of DA antagonists is the drug used (eticlopride vs. pimozide). As eticlopride and pimozide are both relatively selective for DA D_2Rs (Christensen et al. 1984; Werkman et al. 2006), the different drugs employed seems an unlikely source for the different outcomes.

Previous studies have indicated that the effects of DA D_2R antagonists differ under FR 1 and intermittent schedules of reinforcement (Phillips and Fibiger 1979; Tombaugh et al. 1980). Thus, the obtained difference in the effects on responding of DA D_2R antagonist administration and extinction obtained in the present study is likely due to the use of an intermittent schedule of reinforcement rather than the use of different DA D_2R antagonists. That the effects of DA D_2R antagonists resemble extinction under some conditions and not others indicates that the effects of antagonist administration and extinction are not universally equivalent, as might be predicted from the hypothesis that blockade of DA D_2Rs simply interferes with reinforcement.

Previous studies have indicated that reinforcers can also serve a discriminative stimulus function under FR schedules, setting the occasion for a pause in responding (Felton and Lyon 1966; Griffiths and Thompson 1973). In the present study, all stimulus changes (feeder click, light changes, sounds of the pellet dispenser) other than the pellet itself were retained during EXT to more closely model what might occur if the reinforcing, but not discriminative stimulus effects of food delivery were obtunded by DA D_2R blockade. Nonetheless, the obtained increases in response rates during extinction were largely influenced by a decrease in PRP, suggesting that the present effect of extinction was due to the absence of the discriminative stimuli that occasion pauses in responding. Because all stimuli other than those arising from pellet delivery were retained during EXT, the primary discriminative stimulus contributing to the pause was the food pellet itself, and likely those arising from its consumption. These findings suggest that comparisons of drug administration and extinction under conditions that eliminate reinforcer presentation should also consider the possible discriminative function of reinforcer presentation. Further, the view that reinforcer presentation can serve a discriminative function suggests future studies of extinction in which food pellets are delivered independently of responding (e.g., Rescorla and Skucy 1969).

Extinction increased response rates relative to baseline levels, and there were no significant differences in those increases across the three genotypes for either response. Previous results have suggested that a single instance of reinforcing a response can produce a virtually immediate change to asymptotic responding (Skinner 1938, pp. 66–71). In contrast, the extinction of that responding takes much longer to reach asymptotic low levels (ibid, pp. 85–90). These differences in the effects of reinforcement and extinction are mirrored by the present results suggesting that the DA D_2R largely influences the process of reinforcement, as indicated by behavioral economic measures, whereas the process of extinction was largely independent of DA D_2R deletion.

When deprivation levels for the mice were changed with prefeeding, decreases in responding relative to baseline levels were obtained in all three genotypes. The decreases in response rates were equivalent for nose poking, whereas there was a small effect of genotype obtained with lever pressing. These results indicate a lack of substantial involvement of DA D_2Rs in the effects of deprivation and satiation. The contrasting effects of genotype on degree of deprivation and on reinforcing effectiveness (reflected by the behavioral economic measures) indicate a mechanistic distinction between those conditions that establish food as a reinforcer and the reinforcement process itself.

Because food obtained outside the experimental session has been shown to increase estimates of α (Hursh and Silberberg 2008), future studies will examine changes in responding with FR value using a closed economy, in which the entire daily food ration is obtained within the session. Nonetheless, until such time as outside sources of food are shown to differentially alter calculations of reinforcing effectiveness (α), the present findings indicate a clear contribution of the DA D_2R to reinforcing effects of food during conditions of sustained reinforcement.

In addition to lacking DA D_2Rs, D_2R KO mice have a variety of other differences such as reduced dopamine transporter function (Dickinson et al. 1999) and adenosine receptor function (Zahniser et al. 2000). It is therefore possible that other mechanisms recruited during development that result from genetic deletion of the DA D_2R contributed in some way to the obtained results. However, as several behavioral functions related to reinforcement (e.g., deprivation level and extinction) were largely unchanged in

the different lines of mice, such compensatory mechanisms, if involved at all, had a subtle role.

Several studies have shown a link between DA D_2Rs, obesity, and food reinforcement. An inverse relation between D_2R availability in humans (Wang et al. 2001) or expression in rodents (Johnson and Kenny 2010) and body weight has been documented. Additionally, reinforcement with preferred foods is reported to maintain more responding in obese humans with the TaqI A1 allele, which has been shown in other studies to be associated with decreased DA D_2R density (Noble et al. 1991; Thompson et al. 1997), than in individuals without the variant (Epstein et al. 2007). It has been hypothesized that decreased DA D_2R function decreases the effectiveness of reinforcers (Blum et al. 1996), which results in a compensatory over ingestion of food reinforcers (Wang et al. 2001).

What is unclear from those studies is whether decreased D_2R number predisposes an individual to consume more food and gain weight or whether such consumption results in decreased D_2R density. Johnson and Kenny (2010) showed that overconsumption of a high-fat and sugar diet increased body weight and reduced striatal D_2R density and that lentiviral-induced downregulation of D_2Rs rapidly increased the threshold for brain stimulation reinforcement, interpreted as an indication of a general decrease in effectiveness of reinforcers. Although direct comparisons with the current study are limited by the many differences in the way that the studies were conducted, the finding that the reinforcing effectiveness of food was lower in D_2R KO mice is consistent with the hypothesis. However, there was no evidence of a consequent compensatory overconsumption of food in the current study, as unrestricted feeding weights were similar across genotypes.

In summary, the results of the behavioral economic analysis are consistent with the assertion that D_2R function is involved in the reinforcing effects of food. In contrast, the results obtained with both prefeeding and extinction suggest that D_2Rs are not substantially involved in the effects of deprivation level or extinction. Additionally, the within-session increases in response rate produced by extinction were not mirrored by any of the tested doses of (−)-eticlopride, suggesting a lack of functional equivalence between the two manipulations. Finally, the current study demonstrates the utility of a behavioral economic approach in the assessment of the role of specific receptors in mediating reinforcing effects, an approach that can be extended to the study of the role of other receptor subtypes including specific dopamine receptor subtypes and other reinforcers.

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