Published in final edited form as:

Behav Brain Res. 2010 March 5; 207(2): 508. doi:10.1016/j.bbr.2009.10.020.

Dopamine D4 Receptor (D4R) Deletion in Mice Does Not Affect Operant Responding for Food or Cocaine

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Abstract

In this study we examined the genetic contribution of the D4R in food and cocaine self-administration using D4R mice. Mice were examined for operant responding to food pellets or intravenous cocaine. Compared to wild type mice (D4R^{+/+}), both heterozygous (D4R^{+/-}) and knockout (D4R^{-/-}) mice showed no difference in responding for food or cocaine. Our findings suggest that the D4R is not directly involved in mediating operant response behaviors for food or cocaine.

Keywords

reward; addiction; reinforcement; cognition

Introduction

The Dopamine D4 receptor (D4R) is predominantly located in cortical regions in both pyramidal and GABAergic cells (Mrzljak et al., 1996) and in striatal neurons (Rivera et al., 2002). It has been proposed that D4R act as an inhibitory postsynaptic receptor controlling the neurons of the frontal cortex and striatum (Oak et al., 2000). High density of D4Rs also occurs in hippocampus and thalamus (Mrzljak et al., 1996). The gene encoding for the D4R is highly polymorphic and variations in the VNTR region of the third loop have been associated with substance abuse and addiction (George et al., 1993), drug craving (Hutchison et al., 2003), obesity (Poston et al., 1998), binge eating (Leviton et al., 2004), ADHD (LaHoste et al., 1996) and novelty seeking (Ebstein et al., 1996). Recent studies have shown that the 7-repeat allele of the D4R is associated with prefrontal cortex dependent working memory function

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(Herrmann et al., 2007) and in cortical thinning in regions that play a major role in attention (Shaw et al., 2007). Preclinical studies have also implicated the D4R in processes that regulate food intake such as satiety (Huang et al., 2005) and cue-induced food craving (Sobik et al., 2005). Similarly, recent research suggested distinct mechanisms for D4R modulation of the reinforcing (perhaps via attenuating dopaminergic signaling) and locomotor properties of different psychostimulant drugs (Thanos et al., 2009). Furthermore, previous findings suggest that individuals with D4R polymorphisms might show enhanced reinforcing responses to methylphenidate and amphetamine and attenuated locomotor response to amphetamine (Thanos et al., 2009). and novelty seeking (Dulawa et al., 1999), which is a characteristic associated with the propensity for drug self-administration.

However little is known about the involvement of D4R directly on feeding and/or drug reward processes. To address these questions, we assessed the effects of partial or complete D4R deletion in mice on operant responding for food and cocaine.

Methods & Materials

Animals

All mice (originally obtained from D.K. Grandy) were bred at Brookhaven National Laboratory and generated as previously described (Rubinstein et al., 1997). A total of 27 male adult mice (6-7 months old) were used for the food SA experiment [D4R^{+/+} (N=6), D4R^{+/-} (N=11), D4R^{-/-} (N=10)]. A total of 26 mice were used in the cocaine SA experiment [D4R^{+/+} (N=8), D4R^{+/-} (N=11), D4R^{-/-} (N=7)]. All mice were individually housed and maintained in a controlled room (68-72° F and 40-60% humidity) with a 12-h reverse light-dark cycle (lights off at 0800h). All mice were food-restricted to maintain an 85% body weight of age-matched ad-libitum fed mice during operant training with food. The mice used in the food SA experiment continued on this regimen while those used in for cocaine SA were switched to an ad-libitum regimen after operant training. Experiments were conducted during the dark cycle from 1000 to 1300h. All experiments were conducted in conformity with the National Academy of Sciences Guide for Care and Use of Laboratory Animals (NAS and NRC, 1996) and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

Food Self-Administration

Operant chambers (30 × 25 × 30 cm; Coulbourn Instruments, Allentown, PA) were placed inside sound attenuation cubicles. Each operant chamber contained 2 levers and a food receptacle in between. Animals were tested on a fixed-ratio 1 (FR1) schedule and each session lasted 90 minutes. The left lever was designated as the “active” lever and responses to it led to the delivery of a 20 mg food pellet (Research Diets: New Brunswick, NJ), while the right lever was designated as the “inactive” lever and responses to this did not have any consequences but were recorded. Both levers were situated directly under their respective cue lights and pellet delivery was signaled by the onset of the cue light above the lever. After the food pellet delivery, the cue light turned off and there was a time-out for 30s where no food was available. SA experimental variables were programmed using Graphic State v3.02 software (Coulbourn Instruments, Allentown, PA). Mice were first trained for food reinforced lever responding until responding met baseline criteria adopted from previous SA studies (Caine et al., 1999; Caine et al., 2002). After this training phase mice were tested for food SA for a period of 10 days. Behavioral variables assessed consisted of the number of active and inactive lever presses and number of food pellets delivered.

Cocaine Self-Administration

After the same food training schedule as in the food SA experiment, mice received indwelling catheters in the right jugular vein as previously described (Soria et al., 2005). Mice were then

given a 3 day recovery period following jugular catheter surgery. After the recover period mice were tested on cocaine SA for a period of 10 days. Cocaine SA experiments followed the same protocol and design as food SA experiments with the exception that instead of a food pellet, mice were injected with 1 mg/kg of cocaine delivered at a fixed rate of 0.025ml/s for duration of 2s. Catheter patency was tested using a 50/5mg/kg ketamine/xylazine solution every 3 days and at the end of the 10 day period. Catheters were deemed patent only if mice lost the righting reflex within 3 seconds of the injection. Mice that failed the patency test at any time point were excluded from the study.

Results

Food Self Administration

Lever Responses—For active lever responses a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 230)=0.82$; $p=ns$; figure 1). We did find a significant main effect for Time ($F(9, 230)=4.68$; $p<.001$; figure 1) but no interaction effects ($F(18, 230)=0.64$; $p=ns$). For inactive lever responses a two-way ANOVA showed no significant main effects for Strain ($F(2, 231)=3.99$; $p=ns$) and Time ($F(9, 231)=2.82$; $p=ns$) as well as their interaction ($F(18, 231)=1.56$; $p=ns$).

Number of Pellets—For pellets delivered, a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 226)=1.03$; $p=ns$; figure 2). We did find a significant main effect for Time ($F(9, 226)=6.49$; $p<.001$) but no interaction effects ($F(18, 226)=0.83$; $p=ns$).

Cocaine Self Administration

Lever Responses—For active lever responses a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 224)=1.41$; $p=ns$; Figure 1). We did find a significant main effect for Time ($F(9, 224)=7.12$; $p<.001$) but no interaction effects ($F(18, 224)=0.86$; $p=ns$). For inactive lever responses a two-way ANOVA showed no significant main effects for Strain ($F(2, 224)=1.17$; $p=ns$) and Time ($F(9, 224)=2.19$; $p=ns$) as well as their interaction ($F(18, 224)=0.62$; $p=ns$).

Number of Cocaine Infusions Delivered—For cocaine infusions, a two-way ANOVA showed no significant (D4R) genotype effect ($F(2, 215)=1.00$; $p=ns$; Figure 2). We did find a significant main effect for Time ($F(9, 215)=6.41$; $p<.001$) but no interaction effects ($F(18, 215)=0.55$; $p=ns$).

Discussion

Here we show that the D4R is not involved in modulating the operant reinforcing effects of both natural (food) and drug rewards (cocaine). Our results indicate that mice with lower D4R levels (D4R^{+/-}) or totally lacking D4R (D4R^{-/-}) showed similar behavioral responses as wild-type mice, which suggests that the D4R has limited involvement in food and cocaine consumption behavior.

D4R^{-/-} mice have been shown to have lower dopamine release in reward-related mesolimbic areas of the brain (Thomas et al., 2007). Based on this finding, it would seem that D4R^{-/-} mice would be more vulnerable to the rewarding effects of food and cocaine. Indeed studies have shown that D4R^{-/-} mice are more sensitive to the motor effects of ethanol, cocaine, methamphetamine (Katz et al., 2003; Rubinstein et al., 1997) and amphetamine (Kruzich et al., 2004) compared to wild-type mice. However, aside from this motor hypersensitivity to ethanol, the two genotypes do not differ in alcohol consumption (Falzone et al., 2002). Similarly, even though cocaine induces greater motor effects in D4R^{-/-} than in wild-type mice,

we previously found no difference in conditioned place preference for cocaine between D4R^{-/-} and D4R^{+/+} mice (Thanos et al., 2009). One study reported that D4R^{-/-} mice were more sensitive to the discriminative-stimulus effects of a 10 mg/kg IP cocaine than wild-type mice (Katz et al., 2003). However, this study concluded that these effects were probably not mediated by the D4R (and probably due to changes in D2R and D3R due to D4R deletion) since the D2R/D3R antagonist raclopride (which has negligible affinity for D4R) produced similar changes in discriminative-stimulus behavior to cocaine in both strains (Katz et al., 2003). In addition, the highly selective D4R antagonist L-745,870 did not affect discriminative-stimulus effects of cocaine in rats (Caine et al., 2002; Costanza and Terry, 1998). Finally, a recent study showed that D4R^{-/-} mice do not differ from wild-type mice in measures of impulsivity (Helms et al., 2008). This finding substantiates our results since impulsive behavior is linked to the propensity to seek and consume food (Guerrieri et al., 2008) as well as cocaine (Everitt et al., 2008; Perry et al., 2008).

When taken together with these prior findings, our results suggest that the previously observed hypersensitivity of D4R^{-/-} mice to drugs and alcohol may be specific to motor behavior and may not translate to other behaviors specific to food and drug seeking or consumption. This view however may be limited in part by the non-specificity of the genetic manipulation associated with transgenic models. The D4R has been linked to novelty seeking (Benjamin et al., 1996) and has therefore been suggested play a role in substance abuse disorders. In particular, D4R polymorphisms in humans have been linked to alcohol (George et al., 1993) and opiate abuse (Kotler et al., 1997). Our findings suggest that such predispositions may be due to indirect effects of D4R polymorphisms on other brain mechanisms. Future studies are aimed at evaluating the profile of D2R and D3R in D4R^{-/-} mice will prove useful in validating this view.

Acknowledgments

Support Contributed By: NIAAA (Intramural Research Program, LNI), the NIH IRTA program, the Stony Brook URECA program and the BNL SULI program. We also thank N. Londono for her assistance with the D4 mouse breeding colony.

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Abbreviations used

DA
D4R
D2R
SA
IV

Figure 1.
Mean Lever Responses during Food and Cocaine Self-Administration

Figure 2.
Mean Pellets and Infusions delivered during Food and Cocaine Self-Administration