Increased vulnerability to cocaine in mice lacking dopamine D_3 receptors

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Neuroimaging studies using positron emission tomography suggest that reduced dopamine D_2 receptor availability in the neostriatum is associated with increased vulnerability to drug addiction in humans and experimental animals. The role of D_3 receptors (D_3Rs) in the neurobiology of addiction remains unclear, however. Here we report that D_3R KO ($D_3^{-/-}$) mice display enhanced cocaine self-administration and enhanced motivation for cocaine-taking and cocaine-seeking behavior. This increased vulnerability to cocaine is accompanied by decreased dopamine response to cocaine secondary to increased basal levels of extracellular dopamine in the nucleus accumbens, suggesting a compensatory response to decreased cocaine reward in $D_3^{-/-}$ mice. In addition, $D_3^{-/-}$ mice also display up-regulation of dopamine transporters in the striatum, suggesting a neuroadaptative attempt to normalize elevated basal extracellular dopamine. These findings suggest that D_3R deletion increases vulnerability to cocaine, and that reduced D_3R availability in the brain may constitute a risk factor for the development of cocaine addiction.

etiology of addiction | susceptibility to cocaine | reinforcement | extinction | reinstatement

One of the most challenging issues in drug abuse research is understanding the etiology of addiction (1–3). Identification of neurobiological factors conferring vulnerability to drug use and abuse may provide important therapeutic targets for the development of medications to treat addiction. Cumulative evidence suggests that certain vulnerability traits, such as high impulsivity, stress reactivity, novelty-seeking, negative emotionality, and abnormal structure of the frontostriatal brain system, may predispose humans to drug abuse and addiction (4–8). However, the neurobiological mechanisms by which such traits affect drug-taking and drug-seeking behavior are poorly understood. PET studies suggest that reduced dopamine (DA) D_2 receptor (D_2R) availability in the neostriatum is associated with reduced orbitofrontal cortex functional activity, which is linked to risk for impulsivity and compulsive cocaine administration in both humans (9–12) and nonhuman primates (2, 13). Whether reduced D_2R availability is a determinant or consequence of cocaine abuse remains unclear, however (2, 12–14).

It should be noted that the reduced D_2R availability observed in PET or micro-PET studies is based on the use of D_2R-preferring ligands, such as [^{11}C]raclopride (~10- to 20-fold selectivity for D_2 over D_3). This raises the question of whether striatal D_3Rs are also involved in increased susceptibility to drug-taking behavior. Compared with D_2Rs, which are expressed uniformly throughout the striatum (15), D_3Rs are expressed preferentially in the nucleus accumbens (NAc) shell, islands of Calleja, and olfactory tubercle (16, 17). A recent neuroimaging study with the D_3R-preferring ligand [^{11}C]-PHNO (with 10-fold selectivity for D_3 over D_2) revealed significant reduction in D_3R binding in the dorsal striatum of heavy methamphetamine polydrug users (18). In rodents, a significant reduction in D_2R/D_3R binding, as assessed using [^{11}C]raclopride or [^{18}F]fallypride, was found in the NAc but not in the dorsal striatum and was associated with increased cocaine intake in highly impulsive rats (19) and enhanced cocaine-conditioned preference in rats (20). These findings implicate D_3Rs in vulnerability to enhanced cocaine-taking or cocaine-seeking behavior in rats. However, the use of low-selective D_2/D_3-preferring receptor ligands in the foregoing studies makes it impossible to dissociate the roles of D_2Rs and D_3Rs in vulnerability to drug use and abuse.

Therefore, in the present study, we used D_3R gene-deleted ($D_3^{-/-}$) mice to study whether D_3R deletion alters cocaine-taking and cocaine-seeking behavior during acquisition and maintenance of cocaine self-administration, extinction, and reinstatement of drug-seeking behavior. The purpose of this study was to determine whether D_3R loss is a risk factor for cocaine use and abuse. In addition, we also investigated neuroadapative responses in the mesolimbic DA system in $D_3^{-/-}$ mice to determine whether an increase or a decrease in mesolimbic DA response to cocaine underlies changes in cocaine-taking and cocaine-seeking after D_3R deletion.

Results

D_3R Deletion Increases Cocaine-Taking and Cocaine-Seeking Behaviors. We first compared drug-taking and drug-seeking behaviors between WT and $D_3^{-/-}$ mice during cocaine self-administration, extinction, and reinstatement of drug seeking (Fig. 1). The majority of WT mice (22 of 36) and $D_3^{-/-}$ mice (20 of 32) acquired stable i.v. cocaine self-administration after 5–10 d of training, defined as (*i*) at least 20 infusions per 3-h session; (*ii*) less than 20% variability in daily cocaine infusions across two consecutive sessions; and (*iii*) an active/inactive lever press ratio exceeding 2:1 (21, 22). We initially trained mice with a high cocaine dose (1.0 mg/kg/infusion) to promote acquisition of cocaine self-administration and set the maximum number of infusions at 30 to prevent cocaine overdose. We then lowered the cocaine dose to 0.5 mg/kg per infusion (with a maximum of 50 infusions) to maintain high levels of operant responding during self-administration. Compared with WT mice, $D_3^{-/-}$ mice displayed significantly higher active lever responding during cocaine self-administration (genotype main effect, $F_{1,26} = 6.02$, $P < 0.05$) and significantly higher cocaine-seeking during extinction (genotype main effect, $F_{1,26} = 0.39$, $P > 0.05$; genotype × time interaction, $F_{9,234} = 2.16$, $P < 0.05$) (Fig. 1*A*). In addition, $D_3^{-/-}$ mice displayed delayed extinction responding or extinction-resistance, that is, took longer than WT mice to extinguish drug-seeking behavior. Subsequent cocaine priming (10 mg/kg) induced significant reinstatement of drug-seeking after prolonged extinction in both WT and $D_3^{-/-}$ mice ($P < 0.05$, compared with the last extinction session). There was no significant difference in reinstatement responding between the two strains (Fig. 1*A*). No significant differences were observed

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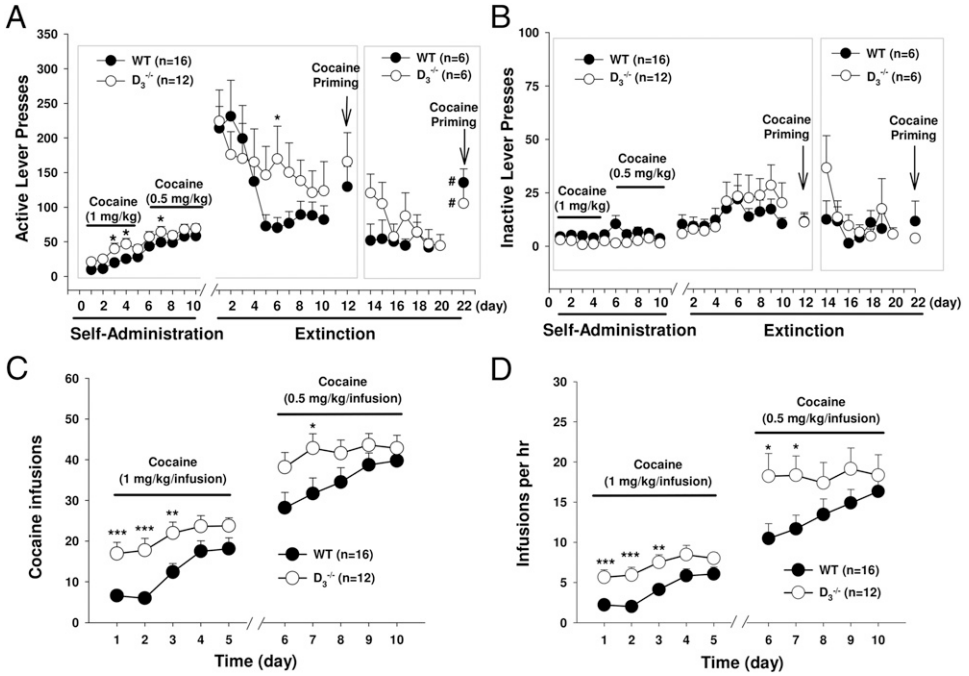

Fig. 1. Cocaine-taking and cocaine-seeking behavior in WT and $D_3^{-/-}$ mice during acquisition and maintenance of cocaine self-administration and during extinction and reinstatement of drug-seeking behavior. (*A* and *B*) Active and inactive lever responding in each phase of the experiment. (*C* and *D*) Total number of cocaine infusions in daily test sessions and infusion rate during the acquisition phase of cocaine self-administration. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with WT control group. $^{\#}P < 0.05$ compared with last day of extinction.

between WT and $D_3^{-/-}$ mice in inactive lever responses during cocaine self-administration ($F_{1,26} = 2.88$, $P > 0.05$) or extinction ($F_{1,26} = 0.295$, $P > 0.05$) (Fig. 1*B*).

We then characterized the changes in cocaine-taking behavior during acquisition and maintenance of cocaine self-administration. Fig. 1 *C* and *D* shows the total number of infusions within daily 3-h sessions and cocaine self-administration rate (infusions per hour, calculated as cocaine infusions over time to the termination of infusions), demonstrating significantly higher cocaine self-administration in $D_3^{-/-}$ mice (Fig. 1*C*; genotype main effect, $F_{1,26} = 7.08$, $P < 0.05$) than in WT mice (Fig. 1*D*; genotype main effect, $F_{1,26} = 5.78$, $P < 0.05$). Fig. S1 shows representative records of cocaine self-administration from day 1 to day 5, illustrating different cocaine self-administration patterns in the two mouse strains. $D_3^{-/-}$ mice displayed faster cocaine-taking behavior with shorter interinfusion intervals compared with WT mice.

To further explore these findings, we examined cocaine self-administration dose–response functions. Fig. 2*A* shows typical inverted U-shaped curves of cocaine self-administration over a range of cocaine doses in WT and $D_3^{-/-}$ mice. Consistent with the foregoing findings, $D_3^{-/-}$ mice displayed a significant upward shift in the cocaine dose–response curve ($F_{1,12} = 15.49$, $P = 0.002$) compared with WT mice. $D_3^{-/-}$ mice also exhibited higher cocaine intake than WT mice (Fig. 2*B*; $F_{1,12} = 20.57$, $P < 0.001$).

D_3R Deletion Increases Motivation to Work for Cocaine Reward. We then examined the effects of D_3R deletion on incentive motivation to perform drug-reinforced operant behavior. Self-administration under progressive-ratio (PR) reinforcement is an operant procedure commonly used to evaluate motivation to obtain a drug or food reward within daily self-administration sessions (23). $D_3^{-/-}$ mice exhibited more cocaine infusions and higher PR breakpoints for cocaine self-administration compared with WT mice (Fig. 2*C*). Two-way ANOVA demonstrated a significant strain difference between WT and $D_3^{-/-}$ mice in the number of cocaine infusions under PR reinforcement ($F_{1,16} = 21.66$, $P < 0.001$), suggesting enhanced motivation to work for cocaine reward in $D_3^{-/-}$ mice.

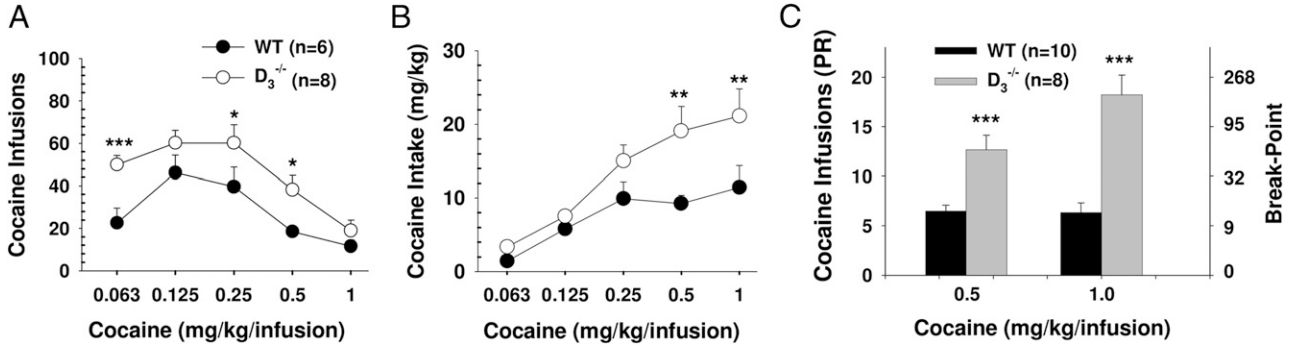

Fig. 2. Cocaine self-administration under different cocaine doses (*A* and *B*) and PR reinforcement conditions (*C*) in WT and $D_3^{-/-}$ mice. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with WT.

D_3R Deletion Increases Sucrose-Taking and Sucrose-Seeking Behavior. To study whether such enhanced cocaine-taking and cocaine-seeking behavior generalizes to nondrug reward-guided behavior, we examined the effects of D_3R deletion on oral sucrose-taking and sucrose-seeking. Fig. S2 shows that $D_3^{-/-}$ mice display higher oral sucrose self-administration (Fig. S2*A*; genotype main effect, $F_{1,22} = 4.69$, $P < 0.05$), and active lever responses during last 5 d of self-administration and subsequent extinction (Fig. S2*B*; $F_{1,22} = 4.19$, $P = 0.053$). We also used PR self-administration to evaluate incentive motivation for sucrose reward. Given that breakpoints are derived from an escalating mathematical function, the data tend to violate the assumption of homogeneity of variance. Therefore, breakpoint data were log-transformed before statistical analysis (24). Compared with WT mice, $D_3^{-/-}$ mice displayed higher oral sucrose infusions under PR reinforcement (Fig. S2*C*; $F_{1,14} = 4.68$, $P < 0.05$) and higher PR breakpoint for sucrose reward (Fig. S2*D*; $F_{1,14} = 4.73$, $P < 0.05$).

D_3R Deletion Increases Basal Extracellular DA, but Decreases DA Response to Cocaine. To study whether the enhanced cocaine-taking behavior is correlated with altered DA-elevating response to cocaine, we investigated the effects of D_3R loss on basal and cocaine-enhanced NAc DA. Fig. 3 shows extracellular NAc DA before and after each of three cocaine doses. $D_3^{-/-}$ mice display higher basal (precocaine injection) levels of extracellular NAc DA compared with WT mice (Fig. 3 *A*, *C*, and *E*). Because basal levels of extracellular DA appeared substantially comparable in the three WT and $D_3^{-/-}$ cocaine dose groups, we pooled basal DA levels from all WT mice and from all $D_3^{-/-}$ mice to increase the power for statistical analysis. We found a significantly higher basal level of extracellular DA in $D_3^{-/-}$ mice compared with WT mice (0.660 ± 0.144 nM vs. 0.291 ± 0.057 nM; $P < 0.05$). Because of this difference, we normalized cocaine-induced changes in DA to the percent change over baseline to compare the DA-elevating response to cocaine in WT and $D_3^{-/-}$ mice. We found that the $D_3^{-/-}$ mice exhibited a significantly lower DA-elevating response to cocaine at 3 or 10 mg/kg, but not at 20 mg/kg [3 mg/kg cocaine: genotype main effect, $F_{1,13} = 5.24$, $P < 0.05$ (Fig. 3*B*); 10 mg/kg cocaine: genotype main effect, $F_{1,13} = 1.43$, $P > 0.05$; genotype × time interaction, $F_{8,104} = 3.69$, $P < 0.001$ (Fig. 3*D*); 20 mg/kg cocaine: genotype main effect, $F_{1,13} = 0.28$, $P > 0.05$ (Fig. 3*F*)]. We also examined presynaptic DA release to high K^+ levels. Local perfusion of high concentrations of K^+ into the NAc produced significant increases in presynaptic DA release in both WT and $D_3^{-/-}$ mice (Fig. S3; KCl treatment main effect, $F_{6,72} = 6.51$, $P < 0.001$); however, no significant difference in high K^+-evoked DA release was observed between the two mouse strains (Fig. S3; $F_{1,12} = 0.61$, $P > 0.05$). Fig. S4 shows the placement of microdialysis probes in the NAc.

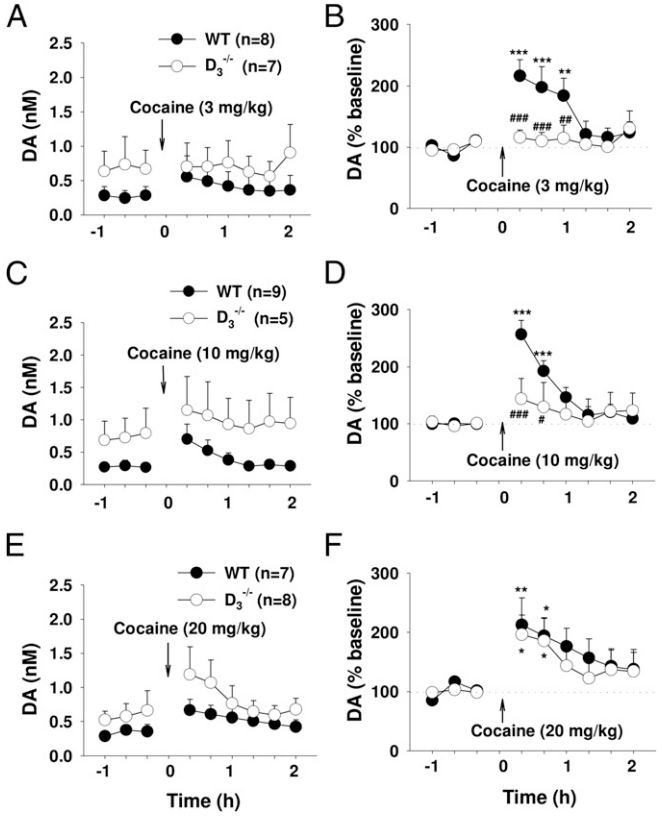

Fig. 3. Extracellular DA levels in the NAc before and after cocaine injection in WT and $D_3^{-/-}$ mice. (*A*, *C*, and *E*) Extracellular DA concentrations (nM) before and after different doses of cocaine administration. (*B*, *D*, and *F*) NAc DA response to cocaine (expressed as percent of precocaine baseline). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared with precocaine baseline. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ compared with WT mice.

D_3R Deletion Increases Basal Level of Locomotion, but Decreases Locomotor-Stimulating Response to Cocaine. To determine whether the lower DA-elevating response to cocaine observed in $D_3^{-/-}$ mice generalizes to other actions of cocaine, we examined the effects of D_3R loss on basal and cocaine-enhanced locomotion. $D_3^{-/-}$ mice displayed significantly higher basal locomotion than WT mice (Fig. S5 *A*, *C*, and *E*). Because of this difference, we normalized cocaine-enhanced locomotion over the 30-min baseline before cocaine (i.e., percent change over baseline), and found significantly lower cocaine-enhanced locomotion in $D_3^{-/-}$ mice [3 mg/kg cocaine: $F_{1,14} = 6.84$, $P < 0.05$ (Fig. S5*B*); 10 mg/kg cocaine: $F_{1,14} = 8.31$, $P < 0.05$ (Fig. S5*D*); 20 mg/kg cocaine: $F_{1,14} = 6.46$, $P < 0.05$ (Fig. S5*F*)].

D_3R Deletion Up-Regulates Dopamine Transporter, but Not Tyrosine Hydroxylase. Finally, we investigated whether D_3R deletion alters DA synthesis and reuptake. Western immunoblot analysis showed significantly higher dopamine transporter (DAT) expression in the striatum, but no significant changes in tyrosine hydroxylase (TH) expression in prefrontal cortex (PFC), midbrain, or striatum of $D_3^{-/-}$ mice (Fig. 4*A*). Further, immunohistochemical assays also demonstrated significantly higher DAT immunostaining in both ventral tegmental area (VTA) and striatum in $D_3^{-/-}$ mice compared with WT mice (Fig. 4 *B* and *C*), but no significant difference in TH immunostaining in these brain regions (Fig. S6).

Discussion

Taken together, our findings indicate that D_3R deletion causes an increase in vulnerability to cocaine, manifested as enhanced cocaine-taking during acquisition and maintenance of cocaine self-administration and enhanced motivation for cocaine-seeking during PR cocaine self-administration and early extinction. This increased vulnerability to cocaine generalizes to sucrose-taking and sucrose-seeking behavior, accompanied by a decreased NAc DA-elevating (and locomotor-stimulating) response to cocaine coincident with increased basal levels of extracellular DA (and locomotion) in $D_3^{-/-}$ mice. These findings suggest that enhanced cocaine-taking behavior could be a compensatory response to reduced cocaine reward after D_3R deletion, and thus reduced D_3R availability might constitute a risk factor (or biomarker) for the development of cocaine addiction.

Previous studies suggest that presynaptic D_2Rs play a major role in regulating presynaptic DA release (25, 26). Our present data demonstrate that D_3R deletion causes a significant (twofold) increase in basal levels of extracellular NAc DA, suggesting that presynaptic D_3Rs also play an important role in controlling presynaptic DA release, consistent with reports of significantly increased extracellular DA in the NAc or dorsal striatum in $D_3^{-/-}$ mice (27–29). This increase in basal extracellular DA might explain the increased basal locomotion observed in the present study and in previous studies (28–31).

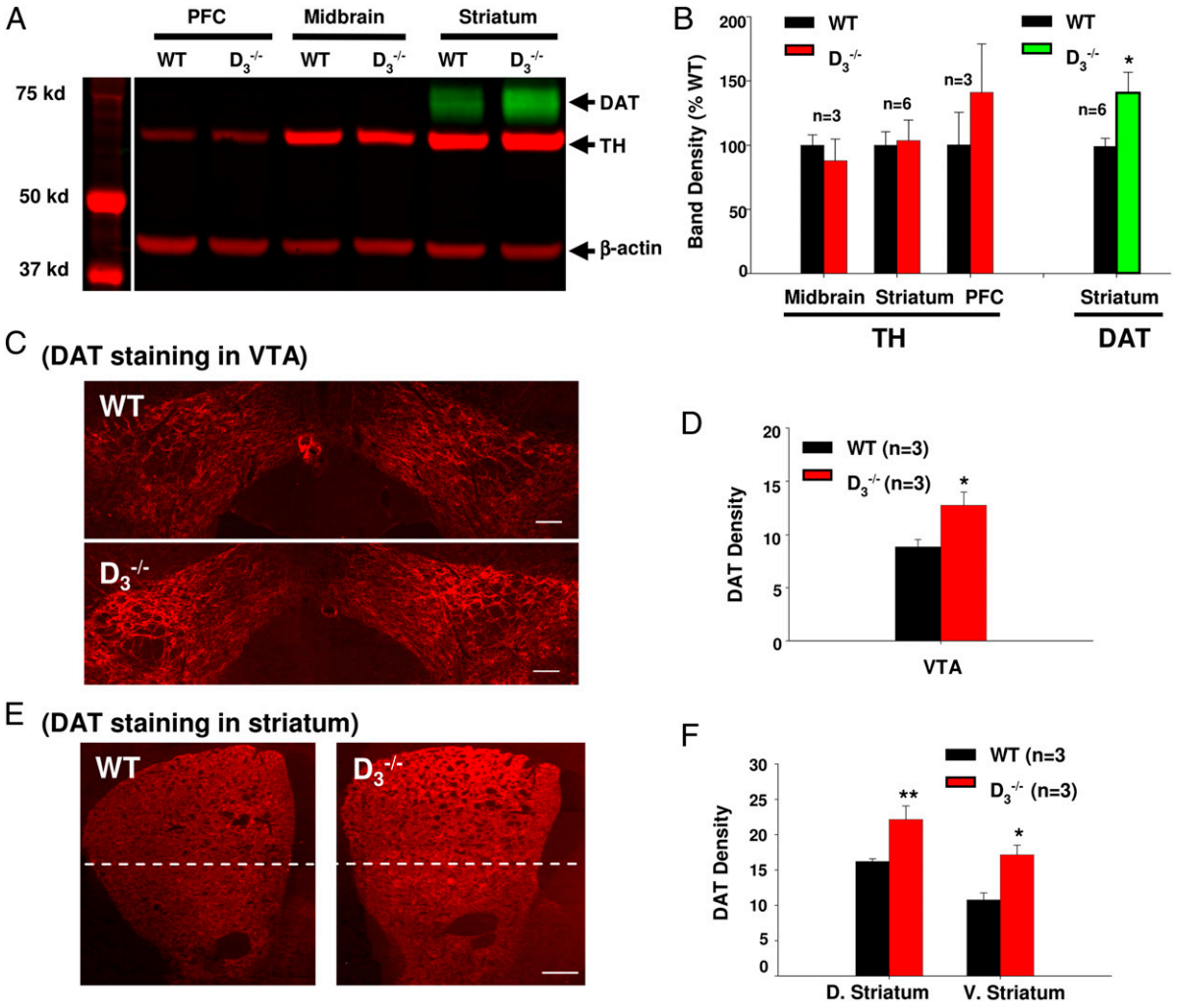

Fig. 4. D_3R deletion up-regulates DAT, but not TH, expression in the striatum. (*A*) Representative immunoblot showing molecular sizes and densities of DAT, TH, and β-actin in PFC, midbrain, and striatum of WT and $D_3^{-/-}$ mice. (*B*) Mean densities (normalized first to β-actin and then to WT mice) of TH and DAT in these brain regions. (*C* and *E*) Representative DAT immunostaining in the VTA or striatum of WT and $D_3^{-/-}$ mice. (*D* and *F*) Mean DAT densities in the VTA or striatum of WT and $D_3^{-/-}$ mice. D. Striatum, dorsal striatum; V. Striatum, ventral striatum. (Scale bars: 100 μm in *C*; 250 μm in *E*). $^*P < 0.05$; $^{**}P < 0.01$ compared with WT mice.

Along with elevated extracellular DA, $D_3^{-/-}$ mice also displayed increased DAT expression, but not TH expression, in the striatum (including NAc) and VTA. We interpret the present DAT up-regulation as a neuroadaptative consequence of enhanced basal extracellular DA after D_3R deletion. This is congruent with a previous report of increased striatal DAT mRNA expression and DA reuptake, but not TH-mRNA and protein expression, in $D_3^{-/-}$ mice (30). In addition, $D_2^{-/-}$ mice also display increased basal levels of extracellular DA and DAT up-regulation (25, 26), suggesting that similar neuroadaptations occur in $D_2^{-/-}$ mice.

Another important finding is that D_3R deletion attenuates the NAc DA response to cocaine. We define the NAc DA response to cocaine as cocaine-induced changes in DA over baseline (i.e., percent of baseline). This is important because the rate of change in extracellular DA is positively correlated with a drug's rewarding efficacy and addictive potential; the faster the increase in extracellular DA, the higher the drug-induced reward and the psychomotor stimulation (32–34). In light of this, reduced DA elevation to cocaine suggests reduced cocaine reward in $D_3^{-/-}$ mice. This reduced DA-elevating response is cocaine dose-dependent and surmountable by increasing doses of cocaine, which may induce animals toward enhanced drug-taking and drug-seeking behavior. $D_3^{-/-}$ mice also exhibit lower locomotor stimulation to cocaine due to increased basal locomotion. This is consistent with a recent report that $D_3^{-/-}$ mice are deficient in locomotor sensitization to chronic morphine (35). Taken together, these data suggest that D_3R deletion causes a reduction in the action of cocaine on DA and DA-related functions. We note two contrary reports of enhanced locomotor response in $D_3^{-/-}$ mice to a low dose of cocaine (31) and to cocaine-associated cues (36). However, in those studies, only total locomotor activity was measured, and the cocaine-induced percentage change in locomotion over baseline was not compared as in the present experiments.

Based on the foregoing, we interpret the enhanced cocaine-taking behavior that we observed as a compensatory response to reduced cocaine reward in $D_3^{-/-}$ mice. We note that an upward shift in the dose–response curve is usually interpreted as an increase in pharmacologic action, but here we interpret the upward shift of the cocaine dose–response curve in $D_3^{-/-}$ mice as a reduction in cocaine reward, because in the dose–response curve descending limb in which most cocaine doses were tested, cocaine-taking behavior was negatively correlated to cocaine dose and (implicitly) cocaine reward strength. Higher cocaine-taking behavior maintained by lower doses of cocaine is generally explained as a compensatory response to lower cocaine reward (37). A similar negative correlation between reward-taking and NAc DA signaling has been reported in other studies; for example, human cocaine addicts exhibit compulsive drug-taking and drug-seeking

while exhibiting diminished striatal DA-elevating responses to cocaine or methylphenidate compared with normal control subjects (10, 33). In rodents, volitional drug-seeking behavior is triggered by phasic decreases in NAc DA, whereas phasic increases in NAc DA are correlated with satiation (38). The present finding that D_3R deletion decreases cocaine reward is consistent with previous findings that D_3R activation potentiates, whereas D_3R antagonism attenuates, cocaine's rewarding efficacy (39–42). We note that a compensatory hypothesis explains only the descending limb, not the ascending limb, of the self-administration dose–response curve. There is no compelling hypothesis to explain why self-administration is positively correlated with cocaine dose on the ascending limb. Given the very low cocaine doses on this limb ($\leq$0.025 mg/kg), one possibility is that the enhanced self-administration may reflect enhanced cocaine-seeking, not cocaine consumption. This is consistent with our finding that $D_3^{-/-}$ mice exhibit higher cocaine-seeking behavior during PR cocaine self-administration and during extinction.

Increased PR breakpoints have previously been interpreted as reflecting increased cocaine reward, given that breakpoints are cocaine dose-dependent within a certain dosage range (23, 42). We note that higher PR breakpoints for cocaine are associated with lower DA elevation to cocaine in $D_3^{-/-}$ mice (the present study) and rats (24), suggesting that a compensatory response may occur in $D_3^{-/-}$ mice under PR reinforcement owing to a reduced DA-elevating response to cocaine. Because PR breakpoint measures the maximal work amount or motivation to obtain cocaine reward during daily self-administration sessions (23, 43), higher breakpoints observed in $D_3^{-/-}$ mice suggest higher drug-seeking behavior. This is consistent with our findings in early extinction in $D_3^{-/-}$ mice. We are very mindful of the fact that changes in operant responding for drug reward under fixed ratio (FR) reinforcement often move in reciprocal fashion to a PR breakpoint, but we also note that this is not always the case. Olsen et al. (43) pointed out that FR responding reflects consummatory behavior, whereas PR responding reflects appetitive responding, and they cite many examples of FR and PR responding moving independently of one another. The mechanisms underlying increased incentive motivation for cocaine-seeking are unclear. Given that extinction responding is largely maintained by drug-associated cues (44), which also stimulate DA release in the NAc and amygdala (45), it is possible that D_3R deletion-induced increases in basal extracellular DA may blunt cue-induced DA release and thus enhance drug-seeking behavior. This attenuated DA-elevating response to cocaine or cocaine-associated cues also may explain higher sucrose-taking and sucrose-seeking behavior in $D_3^{-/-}$ mice, given that a similar DA mechanism has been proposed to underlie natural rewards (46). We are also very mindful that the present findings in $D_3^{-/-}$ mice are seemingly contrary to our previous findings that pharmacologic blockade of D_3Rs attenuates cocaine-taking and cocaine-seeking behavior (42, 47, 48) and shifts the cocaine dose–response curve downward (48). This could be related to neuroadaptations occurring in $D_3^{-/-}$ mice, which attenuate actions produced by D_3R loss itself.

Whatever the mechanisms underlying D_3R loss-induced increase in drug-taking and drug-seeking behavior, the present findings are consistent with previous reports that $D_3^{-/-}$ mice exhibit enhanced acquisition of conditioned place preference to cocaine (49) or amphetamine (31), delayed conditioned place preference extinction (50), increased behavioral sensitivity to concurrent stimulation of D_1 and D_2 receptors (31), and increased gene (c-fos and dynorphin) expression in striatum to cocaine (51). All of these findings support a conclusion that D_3R deletion leads to enhanced susceptibility to cocaine use and abuse. This conclusion appears to conflict with previous reports that cocaine overdose or chronic cocaine administration up-regulates D_3R expression in the NAc in cocaine addicts or experimental animals (52–56). However, we point out that our finding that D_3R deletion causes an increase in cocaine-taking and cocaine-seeking was observed during the acquisition and maintenance of cocaine self-administration and early extinction, whereas D_3R up-regulation in the aforementioned studies was observed after prolonged abstinence (30-45 d of withdrawal from the last cocaine administration), not during self-administration or within 7 d of withdrawal (53, 55, 56). Moreover, findings of D_3R up-regulation were based on the use of low-selective D_3/D_2 receptor ligands, such as [^{3}H]-7-OH-DPAT and [^{125}I]-7-OH-PIPAT, and no control experiments were conducted to rigorously identify the receptor-binding specificity of such ligands (52, 53, 55). Finally, the functional significance of such D_3R up-regulation in relapse to drug-seeking is unclear, given that activation of brain D_3Rs by 7-OH-DPAT or PD-128907 neither reinstates drug-seeking behavior nor alters cocaine-induced reinstatement of drug-seeking behavior (57–59). This is consistent with our finding that cocaine priming produces similar reinstatement in WT and $D_3^{-/-}$ mice.

In conclusion, our present findings demonstrate that DA D_3R deletion produces enhanced cocaine-taking and cocaine-seeking behaviors. This increased vulnerability to cocaine is associated with a decreased DA-elevating response to cocaine. Presynaptic D_3R loss-induced increase in basal DA release appears to play a central role in mediating these behavioral changes.

Materials and Methods

Animals. Male WT and D_3R KO ($D_3^{-/-}$) mice with a C57BL/6J genetic background were bred at the National Institute on Drug Abuse from three $D_3^{+/-}$ breeding pairs purchased from Jackson Laboratory. All mice used in the experiments were matched for age (8–14 wk) and weight (25–35 g). More details are provided in *SI Materials and Methods*.

Intravenous Cocaine Self-Administration. Intravenous catheterization surgery and cocaine self-administration have been described previously (22). Details are provided in *SI Materials and Methods*.

Oral Sucrose Self-Administration and Locomotor Activity. Experimental methods are detailed in *SI Materials and Methods*.

In Vivo Microdialysis. Intracranial guide cannula implantation surgery and in vivo microdialysis procedures were as described previously (22). Basal levels of extracellular NAc DA together with DA response to cocaine were compared in WT and $D_3^{-/-}$ mice, as described in *SI Materials and Methods*.

Immunoblot and Immunohistochemistry Assays. We first used a Western blot assay to compare DAT and TH expression in the PFC, striatum, and midbrain between WT and $D_3^{-/-}$ mice. We then used fluorescent immunochemistry to compare expression of DAT and TH in both striatum and VTA. Methods are described in detail in *SI Materials and Methods*.

Data Analysis. All data are presented as mean ± SEM. One-way ANOVA was used to analyze the difference between WT and $D_3^{-/-}$ mice in terms of basal levels of locomotion and extracellular DA and cocaine-taking behavior, and in terms of density of TH and DAT blotting or staining. Because breakpoint values increase exponentially (23), original breakpoint data were subjected to a logarithmic transformation before data analysis. Two-way ANOVA for repeated measures over time was used to analyze the difference between WT and $D_3^{-/-}$ mice with respect to cocaine-taking and cocaine-seeking and locomotor and extracellular DA responses to cocaine or high concentrations of K^+. Individual group comparisons were carried out using the Student–Newman–Keuls method.

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