Behavioral/Systems/Cognitive

Expression of Mutant NMDA Receptors in Dopamine D_1 Receptor-Containing Cells Prevents Cocaine Sensitization and Decreases Cocaine Preference

Carrie L. Heusner[1] and Richard D. Palmiter[1,2]

[1]Department of Biochemistry and [2]Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195

The interaction of dopamine and glutamate in limbic brain regions mediates behaviors associated with psychostimulants, which act in part to increase dopamine signaling at both D_1 receptors (D1Rs) and D_2 receptors. Many addictive behaviors are a result of learned associations, and NMDA receptor activation has been shown to be important for these behaviors. We hypothesized that if NMDA receptor activation in dopamine receptor-containing cells is required for the addictive properties of psychostimulants, then mice with reduced NMDA receptor activity in D1R-containing cells would have attenuated long-term behavioral changes to these drugs. We generated a mouse line in which D1R-containing cells express an NR1 NMDA receptor subunit containing a mutation in the pore that reduces calcium flux. Mice expressing the mutant NMDA receptors in D1R-containing cells have normal basal activity and display similar increases in locomotor activity when treated with acute amphetamine or cocaine. However, the mutant mice fail to display locomotor sensitization to repeated cocaine administration. In addition, these mice also have a decreased ability to form a conditioned place preference to cocaine. These data suggest that intact NMDA receptor signaling in D1R-containing cells is required for the manifestation of behaviors associated with repeated drug exposure.

Key words: **dopamine; NMDA receptor; conditioned place preference; cocaine; sensitization; transgenic**

Introduction

Psychostimulants act in part by increasing dopamine neurotransmission in key brain regions (Reith et al., 1986; Ritz et al., 1987), causing both locomotor activity and intracellular modifications (Kelly et al., 1975; Clarke et al., 1988; Delfs et al., 1990; Graybiel et al., 1990; Konradi et al., 1994). Repeated treatments with psychostimulants can cause both long-term cellular changes and elicit specific behaviors that are believed to mimic drug-seeking behaviors in humans (Post et al., 1992; Robinson and Berridge, 1993; Nestler, 2001). The persistent molecular effects of psychostimulant exposure produce behavioral sensitization that is revealed by increased locomotion with repeated exposure to the drug (Segal et al., 1980; Robinson and Becker, 1986). Psychostimulants also have rewarding effects in animals; they can learn to associate the hedonic effects of cocaine or amphetamine with the environment in which they were administered (Spyraki et al., 1982; Pitts and Marwah, 1987), developing a conditioned preference for that location.

In addition to affecting dopamine neurotransmission, there is substantial evidence that repeat treatment with psychostimulants also affect glutamate signaling. After repeated cocaine administration, basal extracellular glutamate levels are depressed (Baker et al., 2003; Kozell and Meshul, 2003); however, the amount of glutamate released is significantly increased compared with a single cocaine treatment (Hotsenpiller and Wolf, 2003; McFarland et al., 2003). Additionally, pharmacological experiments have shown a requirement for intact glutamate signaling in the development of behavioral sensitization and place conditioning. Pretreatment of rodents with NMDA receptor antagonists has been shown to block sensitization to cocaine or amphetamine (Karler et al., 1989, 1994; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993; Wolf and Jeziorski, 1993; Haracz et al., 1995) and the development of a conditioned place preference to cocaine (Kim et al., 1996; Harris and Aston-Jones, 2003), implicating a role for NMDA receptor-mediated signaling in the long-term changes that occur during repeated drug administration. Although many studies have implicated specific brain regions such as the ventral tegmental area (VTA) (Kalivas and Alesdatter, 1993; Harris and Aston-Jones, 2003; Licata et al., 2004) and nucleus accumbens (NAc) (Khan and Shoaib, 1996) as being critical brain regions involved in glutamatergic control of behavioral sensitization, the exact cell types in which glutamate receptor activation is required remain unknown.

To study the role of NMDA receptors in drug-induced behaviors, we generated a line of mice that expresses mutant NR1 NMDA receptor subunits in neurons expressing the D_1 receptor (D1R). The asparagine to arginine mutation at codon 616 (N616R) is in the ion pore, and when expressed in cultured cells,

Received April 14, 2005; revised June 3, 2005; accepted June 10, 2005.

This work was supported by United States Public Health Service Grant T32-DA07278 (to C.L.H.) from the National Institute on Drug Abuse. We thank Ed Ziff and Gerald Rameau for NR1 cDNAs, Glenda Froelick for assistance with histology, Serge Luquet for assistance with RT-PCR, and Tom Hnasko and Elena Chartoff for comments on this manuscript.

Correspondence should be addressed to Richard Palmiter, Howard Hughes Medical Institute, Box 357370, University of Washington, Seattle, WA 98195. E-mail: palmiter@u.washington.edu.

DOI:10.1523/JNEUROSCI.1474-05.2005

 0270-6474/05/256651-07$15.00/0

it decreases calcium flux through NMDA receptors (Rameau et al., 2000). By restricting expression of this mutant subunit to neurons expressing the D1R, we generated a model of reduced NMDA receptor signaling in cells that receive both dopaminergic and glutamatergic input, unlike models of systemic or local treatment with NMDA antagonists, or a global downregulation of NMDA receptor expression (Mohn et al., 1999). This includes striatal medium spiny neurons, the most abundant D1R-containing cell type, as well as some cortical and hippocampal neurons.

Materials and Methods

Generation of mutant mice

The targeting construct contained 7.0 kb of 129/Sv-derived *Drd1a* receptor genomic sequence. The *Nco*I site that contained the initiation codon of the *Drd1a* locus was converted to a *Bgl*II site, effectively destroying the initiation codon. The NR1 cDNA (gift from Gerald Rameau, New York University, New York, NY), containing an open reading frame with a hemagglutinin (HA)-epitope tag at a *Hinc*II site 27 amino acids from the N terminus, the N616R mutation, and a polyadenylation sequence, was cloned between this *Bgl*II site and a downstream *Hin*dIII site, replacing ~130 bp of the endogenous *Drd1a* gene. The resulting targeting construct had ~3 kb of genomic *Drd1a* DNA 5′ and ~4 kb of *Drd1a* DNA 3′ to the mutant NR1 cDNA. A simian virus 40–neomycin resistance (Neo) cassette that is flanked by loxP sites was inserted immediately after the NR1 cDNA to allow selection in embryonic stem cells.

The linearized targeting vector was electroporated into V6.4 embryonic stem cells derived from a cross of 129/Sv and C57BL/6 mice. G418-resistant clones were expanded and screened by Southern blotting. For Southern blots, genomic DNA was digested with *Hin*dIII and probed with a 300 bp fragment of DNA from the *Drd1a* receptor locus immediately 5′ to the targeting vector (see Fig. 1*A*). Homologous recombination resulted in a product that was ~4 kb larger than the wild-type (WT) product. Of 360 clones screened, six were correctly targeted, and one gave germline transmission. Progeny carrying the mutant allele were genotyped using PCR primers to detect the presence or absence of the neomycin resistance gene (5′-GCTATTGGGCGAAGTGCCGG-3′ and 5′-GGCAAGCAGGCATCGCCATG-3′). Southern blot on genomic DNA from Neo-positive pups confirmed the PCR results. Additional genotyping was performed using primers flanking the inserted sequence (5′-GACCAGGAAGAGGCCCCAGACT-3′ and 5′-GTCACCTTGGACCGCAGGTGTC-3′) and a primer from the NR1 cDNA (5′-CATGCTTGCGCGTGCTCAGCAC-3′). The mice containing the targeted mutation *Drd1a* $^{Grin1\text{-}N616R}$ (designated D1R-NR1^{m} mice) were maintained on a mixed C57BL/6 × 129/Sv background. Control animals were littermates of D1R-NR1^{m} mice that did not contain the targeted mutation (designated WT mice).

Maintenance and care of animals

Mice were housed in a modified specific-pathogen-free facility under a 12 h light/dark cycle. Food (Purina 5053; Purina Mills, St. Louis, MO) and water were available *ad libitum*. During the 2 d locomotor experiment, food and water were available *ad libitum*; however, during all other experimental procedures, food and water were not available for the length of the test (3–6 h). All procedures were conducted in accordance with guidelines established by the National Institutes of Health and the University of Washington Animal Care Committee.

Reverse transcription-PCR

Mice were killed by lethal injection with sodium pentobarbital, and the dorsal and ventral striatum were rapidly dissected from the brain. Tissue was sonicated, and RNA was isolated using Trizol reagent. Primers (5′-CACCTGCTGACATTCGCCC-3′ and 5′-CAGGGCCATCTGTATGGCG-3′) flanking the HA tag in the NR1 cDNA were used to amplify both the wild-type product and the mutant product, which is 39 bp larger than the wild-type product.

Immunohistochemistry

Mice were killed by lethal injection with sodium pentobarbital and transcardially perfused with PBS followed by 4% paraformaldehyde. Brains were removed and postfixed overnight in paraformaldehyde and then transferred to 70% ethanol for 24 h. Brains were then dehydrated and embedded in paraffin and cut on a microtome in 8 μm sections. Sections were mounted on slides and rehydrated before staining. Antigen retrieval was performed by boiling slides for 8 min in 1 mM EDTA, pH 7.5, and then allowing them to cool for 1 h. Sections were quenched with 3% hydrogen peroxide for 20 min and blocked in 3% normal donkey serum and 0.05% sodium azide in TBS-Triton (20 mM Tris, pH 7.5, 500 mM NaCl, 0.1% Triton-X). Sections were incubated in rabbit anti-HA (1:500; Zymed, San Francisco, CA) at 4°C overnight, washed, and then incubated in donkey anti-rabbit cyanine 3 (1:250; The Jackson Laboratory, Bar Harbor, ME) for 2 h at room temperature. Slides were washed and coverslipped, and fluorescent labeling was visualized.

Drugs

Amphetamine (Sigma, St. Louis, MO) and cocaine (Sigma) were dissolved in PBS (10 mM phosphate, 150 mM NaCl, pH 7.0). Vehicle treatment was PBS alone. All treatments were by intraperitoneal injection at 10 μl/g body weight.

Behavioral analysis

Locomotor behavior. Locomotor activity was measured in transparent Plexiglas cages (40 × 20 × 20 cm) set in activity chambers equipped with four infrared beams set 8.8 cm apart (San Diego Instruments, San Diego, CA). The number of consecutive beam breaks is reported as ambulations.

Acute drug response. Animals were allowed to acclimate to the testing chambers for 3 h, after which they received an injection of either vehicle or drug. Locomotor activity in response to treatment was then recorded for an additional 3 h. Animals were then removed from the testing chambers and returned to their home cages.

Sensitization. Animals were placed in locomotor activity chambers and allowed to acclimate for 1.5 h, at which point they received either vehicle treatment (day 0) or cocaine (20 mg/kg body weight) (days 1–5). Response to drug was measured for 2.5 h, after which mice were returned to their home cages and the chambers were cleaned.

Place conditioning. The place-conditioning apparatus consisted of two testing chambers (20 × 20 cm) that differ in color, floor texture, and odor and a middle shuttle chamber. Mice were prescreened by placing them in the middle chamber and allowing them to freely explore the chambers for 15 min. Each session was videotaped, and the time spent in each section of the apparatus was analyzed by EthoVision software (Noldus Information Technology, Wageningen, The Netherlands). Any animal that spent >60% of the total time in either side chamber or >40% of total time in the middle chamber was removed from the study. The remaining mice were assigned a drug-paired side based on their initial preferences so that the average amount of time spent on either the drug- or saline-paired side in each genotype was 50% of the total spent on both sides minus time spent in the middle chamber. Mice were injected with PBS and confined to the nondrug side in the morning for 30 min; 4 h later, they were injected with drug and confined to the other side for 30 min. There were 3 consecutive pairing days. The following day, mice were again allowed to explore the chamber for 15 min, and the time spent in each chamber was again analyzed using EthoVision. Data are presented as the percentage of time spent on the drug-paired side before pairing sessions (pretest) and after 3 d of pairing sessions (posttest). Percentages are presented rather than total seconds because this is the best representation of a preference for the drug-paired side as opposed to the saline-paired side.

Data analysis

Dose–response was analyzed using two-way ANOVA (genotype-by-dose) followed by Fisher's *post hoc* tests. Behavioral sensitization was analyzed using two-way ANOVA (genotype-by-treatment) with repeated measures on treatment day. Significant main effects were followed by Fisher's *post hoc* tests. Place-conditioning results were analyzed using paired *t* tests between pretest and posttest scores.

Results

Generation of mutant mice

We constructed a targeting vector in which the coding sequence of the *Drd1a* gene was interrupted by an NR1 cDNA open reading

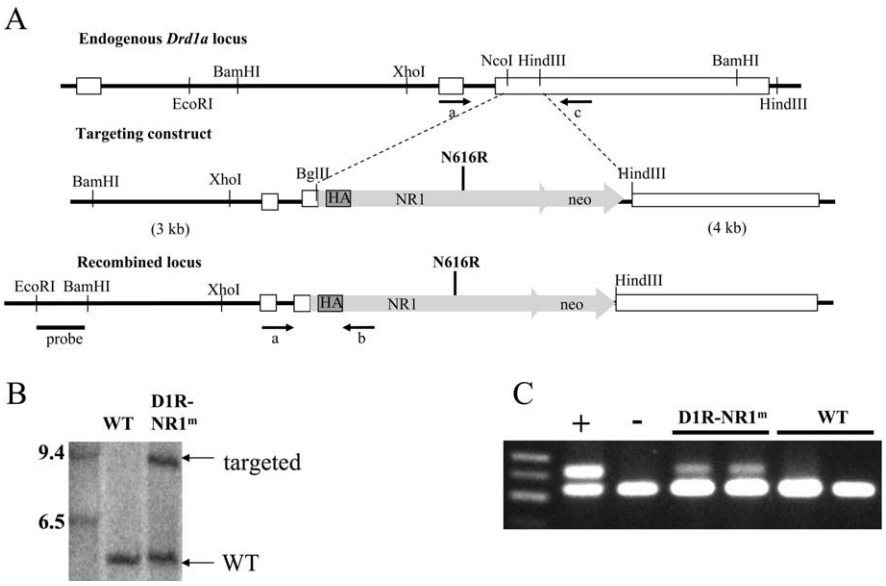

Figure 1. Generation of D1R-NR1^m mice. ***A***, Targeting strategy for D1R-NR1^m mice showing endogenous *Drd1a* locus (top) targeting vector containing NR1 cDNA (middle) and recombined *Drd1a* locus (bottom). Primers for genotyping the wild-type allele (a and c) and the targeted allele (a and b) are indicated. ***B***, Southern blot on DNA from wild-type and mutant mouse. Genomic DNA was digested with *Hin*dIII and hybridized with a probe from the *Drd1a* locus located immediately 5′ to the targeting vector. ***C***, RT-PCR using PCR primers that flank the HA tag. Positive control is a plasmid containing the targeting vector, and the negative control is a plasmid containing the NR1 cDNA without the HA tag.

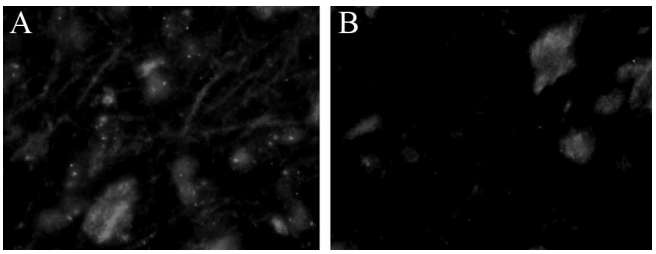

Figure 2. Expression of HA-tagged NR1. ***A***, Expression of HA-tagged NR1 in a the dorsal striatum of a D1R-NR1^m mouse. ***B***, Dorsal striatum of a wild-type mouse with no HA-positive staining in neuronal processes.

frame containing an HA-epitope tag, the N616R mutation and a polyadenylation sequence, and a neomycin resistance gene (Fig. 1*A*). Homologous recombination of this vector in embryonic stem cells resulted in the replacement of one *Drd1a* receptor allele by the mutated NR1. Southern blot analysis on mouse tissue revealed a targeted band that is ~4 kb larger than the wild-type band (Fig. 1*B*). Reverse transcription-PCR (RT-PCR) using primers that flank the HA tag confirmed that mutant mRNA, 39 bp larger than wild-type mRNA, was transcribed in the striatum of D1R-NR1^m mice (Fig. 1*C*). Cerebellar samples from both wild-type and D1R-NR1^m mice did not reveal the top band representing the HA tag, and samples with no reverse transcriptase added produced no bands (data not shown).

Expression of HA-tagged NR1 in brain tissue

To confirm that the mutant protein was expressed, brain sections through the striatum were stained with an antibody to HA. In D1R-NR1^m mice, HA-positive neuronal processes were observed throughout the striatum (Fig. 2*A*), as well as sparse staining in the cortex and hippocampus (data not shown). This staining pattern was absent in wild-type mice (Fig. 2*B*).

Basal locomotor activity

To determine whether D1R-NR1^m mice displayed any abnormalities in basal locomotion, locomotor behavior in response to a novel environment was recorded. When placed in a novel environment for 3 h, both wild-type and D1R-NR1^m mice displayed exploratory behavior that gradually decreased over the course of the experiment (Fig. 3*A*). When mice were housed in the activity chambers for 2 d, both wild-type and D1R-NR1^m mice showed increased activity during the night compared with the day. There was no difference in the number of ambulations between genotypes during either night or day for either of the 2 d (Fig. 3*B*).

Acute drug response

Both wild-type and D1R-NR1^m mice were treated with low to moderate doses of either cocaine or amphetamine to determine whether there was a difference in the acute locomotor response between genotypes. Both groups of mice manifested a locomotor response to amphetamine (3 and 5 mg/kg) and displayed a significant increase in locomotion compared with vehicle treatment at both the 3 and 5 mg/kg doses. Locomotion was not significantly different between genotypes ($F_{(2,88)} = 48.3$) (Fig. 4*A*). Mice were observed for signs of stereotypy (intense grooming, sniffing, or rearing) at 30 min intervals after treatment with amphetamine. At the 3 mg/kg dose, bouts of stereotyped behaviors were followed by bouts of locomotion for the first hour after injection. After the 5 mg/kg dose, bouts of stereotype behaviors persisted through the first 2 h after injection. There was no difference in the types of behaviors or the duration or frequency of the bouts between genotypes (data not shown).

Both wild-type and D1R-NR1^m mice manifested locomotor responses to three doses of cocaine (10, 20, 40 mg/kg) that were not significantly different between genotypes ($F_{(3,104)} = 20.6$) (Fig. 4*B*). Both genotypes were similar at all three doses tested; at 10 mg/kg, neither genotype was significantly different from vehicle, whereas at 20 and 40 mg/kg, both genotypes displayed a locomotor response that was significantly different from vehicle.

Sensitization to cocaine

Glutamate neurotransmission via NMDA receptors is believed to be important for the development of psychostimulant sensitization. Hence, we tested whether D1R-NR1^m mice were able to display locomotor sensitization to repeated treatments with cocaine. Mice were treated with either vehicle (day 0) or cocaine (20 mg/kg) for 5 consecutive days (days 1–5). Wild-type mice significantly increased their locomotor response to cocaine on days 3, 4, and 5 compared with their locomotor response on day 1 ($F_{(5,138)} = 2.4$) (Fig. 5). D1R-NR1^m mice had a small but insignificant increase in the locomotor response to cocaine compared with day 1. Because it has been shown previously that *Drd1a*-null mice do not sensitize to the locomotor-stimulating effects of cocaine (Zhang et al., 2000), we wanted to confirm that the heterozygosity at the *Drd1a* locus in the D1R-NR1^m mice did not contribute the lack of cocaine-induced behavioral sensitization. Using a separate line of mice that are heterozygous at the *Drd1a*

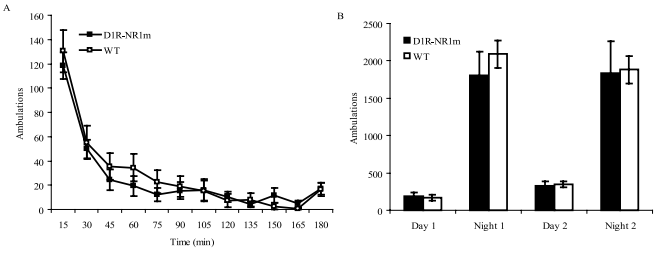

Figure 3. Locomotor activity in wild-type and D1-NR1^m mice. ***A***, Ambulations during 3 h in a novel environment, measured in 15 min increments. ***B***, Ambulations for 2 d by both wild-type ($n = 8$) and D1R-NR1^m ($n = 8$) mice measured in 12 h increments. Error bars represent SEM.

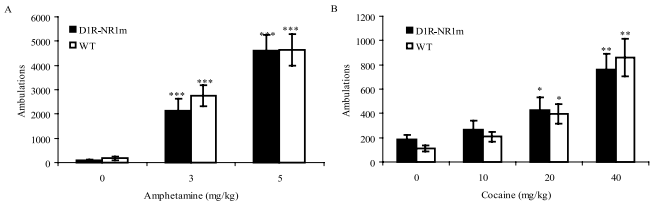

Figure 4. Acute responses to amphetamine and cocaine. ***A***, Ambulations in response to vehicle or amphetamine by both wild-type ($n = 16$) and D1R-NR1^m ($n = 16$) mice. ***B***, Ambulations in response to vehicle or cocaine by wild-type ($n = 14$) or D1R-NR1^m ($n = 14$) mice. $*p < 0.05$; $**p < 0.01$; $***p < 0.005$ compared with vehicle dose for that genotype; two-way ANOVA; Fisher's *post hoc* test. Error bars represent SEM.

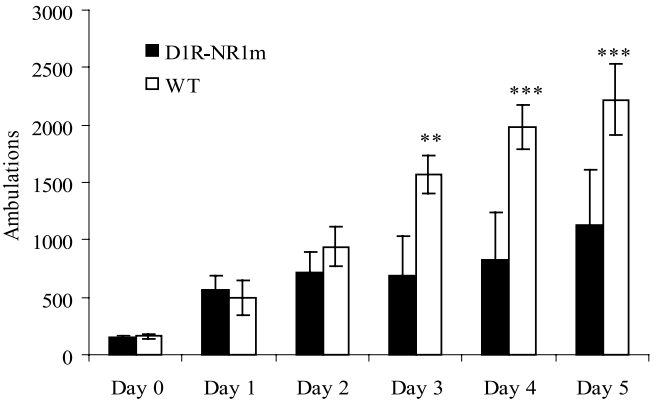

Figure 5. Sensitization to cocaine. Locomotor response to vehicle (day 0) and repeat injection with cocaine (20 mg/kg) (days 1–5) measured for 2.5 h. $**p < 0.01$; $***p < 0.005$ compared with day 1 of the corresponding genotype; two-way ANOVA; Fisher's *post hoc* test (wild type, $n = 12$; D1R-NR1^m, $n = 13$). Error bars represent SEM.

locus and their wild-type littermate controls, we confirmed that heterozygosity at the *Drd1a* locus itself does not prevent the development of behavioral sensitization to cocaine. Like the wild-type littermates of D1R-NR1^m mice, which increased their locomotor response to cocaine by 4.5-fold, mice heterozygous at the *Drd1a* locus displayed an fourfold increase in their locomotor response to cocaine and a significant difference on days 3, 4, and 5.

Place conditioning

Because D1R-NR1^m mice did not sensitize to the locomotor activating effects of cocaine, we tested the rewarding effects of cocaine in a place-conditioning experiment. After 3 d of conditioned pairing with cocaine at 20 mg/kg, the amount of time wild-type mice spent on the drug-paired side was significantly

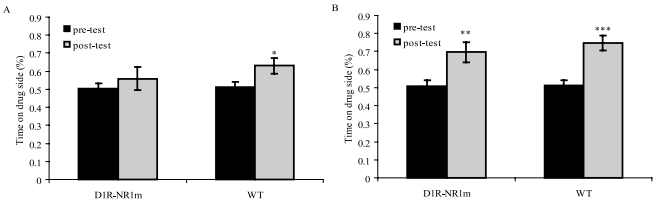

Figure 6. Place conditioning for cocaine. ***A***, Percentage of time spent on drug-paired side before pairing sessions (pretest) and after 3 d of pairing (posttest) for 20 mg/kg cocaine. $*p < 0.05$; paired *t* test (wild type, $n = 12$; D1R-NR1^m, $n = 12$). ***B***, Percentage of time spent on drug-paired side both pretest and posttest after pairings with 30 mg/kg cocaine. $**p < 0.01$; $***p < 0.005$; paired *t* test (wild type, $n = 9$; D1R-NR1^m, $n = 10$). Error bars represent SEM.

greater than the amount of time spent there during the pretest, reflecting a place preference for cocaine. The amount of time spent on the drug-paired side in D1R-NR1^m mice was not significantly different from that spent on that side during the pretest (Fig. 6*A*). However, when pairing was performed using cocaine at 30 mg/kg, both groups manifested a preference for the drug-paired side of the apparatus, suggesting that D1R-NR1^m mice are less sensitive to the rewarding effects of cocaine than wild-type mice (Fig. 6*B*). The total number of seconds spent in the drug-paired, saline-paired, and middle compartments both pretest and posttest for both doses are shown in Table 1. It has been shown that *Drd1a*-null mice, as well as mice that are heterozygous at the *Drd1a* locus, retain the ability to form a conditioned place preference to cocaine (Miner et al., 1995). Therefore, we were not concerned that the blunted place-conditioned preference in D1-NR1^m mice was because of heterozygosity at the *Drd1a* locus.

Discussion

We have shown that expression of a mutant NMDA receptor subunit specifically in neurons that express the D1R does not affect basal locomotion or the acute response to psychostimulants. However, the expression of behaviors that occur after repeated treatment with cocaine, behavioral sensitization and place conditioning, are blunted in D1R-NR1^m mice compared with wild-type controls. These data suggest, as has been found previously in studies using NMDA receptor antagonists, that activation of NMDA receptors is a critical component of the development of behavioral sensitization to cocaine (Karler et al., 1989; Kalivas and Alesdatter, 1993; Wolf and Jeziorski, 1993; Haracz et al., 1995) and cocaine-induced place preference (Kim et al., 1996; Harris and Aston-Jones, 2003). Furthermore, our data allow us to pinpoint neurons that express D1R as being an integral aspect of the synaptic changes that occur during behavioral sensitization and place conditioning.

Several studies have found that pretreatment with NMDA receptor antagonists can prevent behavioral sensitization to cocaine. Systemic treatment with NMDA receptor antagonists such as (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801) have been shown to block behavioral sensitization to either cocaine or amphetamine (Karler et al., 1989, 1994; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993; Wolf and Jeziorski, 1993; Haracz et al., 1995). However, the mechanism by which systemically delivered NMDA receptor antagonists block sensitization is unclear. In addition to blocking NMDA receptors, NMDA receptor antagonists increase extracellular glutamate levels in both the prefrontal cortex and NAc when administered systemically (Moghaddam and Adams, 1998). Thus, with systemic treatment of both cocaine and NMDA recep-

Table 1. Seconds spent in each compartment of the place-conditioning apparatus

Treatment	Cocaine 20 mg/kg		Cocaine 30 mg/kg	
Genotype	WT	D1R-NR1^m	WT	D1R-NR1^m
Pretest (s)				
Drug side	324.9 ± 16.2	322.7 ± 24.9	320.9 ± 18.6	297.2 ± 25.1
Saline side	308.7 ± 16.2	310.9 ± 18.9	306.9 ± 11.0	285.6 ± 17.5
Middle	158.5 ± 13.2	158.5 ± 23.7	164.3 ± 12.6	209.3 ± 26.5
Posttest (s)				
Drug side	397.6 ± 29.8*	358.1 ± 51.4	511.6 ± 25.2***	413.8 ± 43.7*
Saline side	231.8 ± 27.9*	266.2 ± 39.3	175.4 ± 31.9**	231.3 ± 46.0
Middle	148.4 ± 13.8	167.8 ± 27.8	105.2 ± 3.1*	147.0 ± 14.2*

Numbers represent the mean ± SEM number of seconds for the place conditioning at 20 and 30 mg/kg cocaine before pairing sessions (pretest) and after 3 d of pairing (posttest). For cocaine at 20 mg/kg, wild type, $n = 12$, D1R-NR1^m, $n = 12$; at 30 mg/kg, wild type, $n = 9$, D1R-NR1^m, $n = 10$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ compared with pretest score; paired t test.

tor antagonists, it is impossible to know which brain regions are being affected and whether it is the receptor blockade or the increase in glutamate levels that produced the effects. Direct injection of MK-801 into the VTA prevents the development of sensitization to amphetamine (Khan and Shoaib, 1996). The NAc has also been implicated as playing a role in sensitization, because direct injection of non-NMDA receptor antagonists into the NAc blocks the expression of a sensitized response to cocaine (Pierce et al., 1996). These data suggest that modulation of glutamate signaling in the VTA and NAc are important for behavioral sensitization to cocaine.

Behaviors such as sensitization and place conditioning depend on repeated drug exposure. Cues that are presented in the place-conditioning paradigm (color, floor texture, and scent) allow animals to make associations between the experience of receiving the drug and the environment in which the drug is received. Multiple conditioning days are usually required in order for the animal to manifest a drug-induced place preference (Tzschentke, 1998). This indicates that there is likely some amount of synaptic remodeling that is occurring during the repeated pairings that is required for the manifestation of a conditioned place preference. Although some behavioral sensitization to cocaine can occur when animals receive cocaine treatment in their home cages, it is significantly more robust when animals receive treatment in a novel test environment (Haracz et al., 1995), again implying that some associative learning is involved in the behavioral sensitization that we observe in wild-type mice. The influx of calcium via activated NMDA receptors is necessary for many forms of synaptic plasticity that are thought to underlie stable changes in synaptic efficacy and persistent changes in behavior. The N616R mutation in the D1R-NR1^m mice, which lies in the ion pore of the NMDA receptor and retards calcium influx, is likely affecting the synaptic modifications that normally occur during repeated treatment with cocaine. As a result, we see changes in behavioral sensitization and cocaine-induced place preferences, behaviors that depend, in part, on associative learning but no significant deficits in basal locomotor activity or acute psychostimulant-induced activity.

Activation of both D_1 and NMDA receptors has been shown to be involved not only in the behavioral consequences of repeated drug administration, but also on the molecular changes that occur after psychostimulant administration. Within the NAc, induction of ΔFosB, a protein associated with long-term drug exposure (Nestler et al., 2001), appears to occur preferentially in D1R-containing cells (Moratalla et al., 1996; Kelz and Nestler, 2000). Amphetamine-induced expression of c-fos that occurs in dopamine receptor-containing cells after treatment with amphetamine requires activation of NMDA receptors (Konradi et al., 1996). Activation of the transcription factor cAMP response element-binding protein (CREB) is required for the long-term changes that occur during repeated drug administration (Konradi et al., 1994; Robinson and Kolb, 1999; Hyman and Malenka, 2001). It has been shown that activation of CREB requires D1R-mediated phosphorylation of the NR1 subunit, which facilitates NMDA receptor-mediated calcium signaling that is necessary for CREB activation (Dudman et al., 2003). Such intracellular interactions underscore the relationship between D1R and NMDA receptors in the changes that occur with a neuron after repeated psychostimulant exposure. This leads to the hypothesis that a decrease in NMDA receptor-mediated signaling could disrupt the normal changes in gene expression that would occur during a behavioral sensitization paradigm or place conditioning. Without these changes, the behavioral readout would be diminished, as we observe in out D1R-NR1^m mice.

One consideration of the D1R-NR1^m mice is that by using a chronic genetic modulation of NMDA receptors as opposed to a pharmacological antagonism, it is impossible to distinguish between deficiencies in the establishment, as opposed to the expression, of behavioral sensitization. Because the mutant NR1 subunit cannot be removed after cocaine treatment, we do not know whether we are inhibiting the development of sensitization or whether the animals actually are sensitized but cannot express it. The majority of the pharmacological studies have demonstrated a requirement for NMDA receptor-mediated signaling in the establishment of sensitization (Karler et al., 1989; Kalivas and Alesdatter, 1993; Stewart and Druhan, 1993; Wolf and Jeziorski, 1993) while playing less of a role in the expression of sensitization (Karler et al., 1990, 1991). Based on this, we hypothesize that the expression of a mutant NR1 subunit causes the animals to be deficient in the establishment, rather than the expression, sensitization to cocaine; however, we cannot rule out deficiencies in the expression of behavioral sensitization.

By targeting the mutated NR1 subunit to the *Drd1a* locus, we restricted localization only to cells that express the D1R. However, the percentage of dopamine receptor-containing neurons that express only D1R as opposed to either D_2 receptor (D2R) only or both D1R and D2R is unclear. Some studies (Gerfen et al., 1990) conclude that there is very little overlap in the number of neurons that express these receptors. However, others (Surmeier et al., 1992; Aizman et al., 2000) suggest that many, and possibly all, dopamine receptor-containing neurons, in the striatum at least, express both receptor types. If there is a high degree of overlap, we cannot conclude that some dopamine receptor-containing cells are more important than others in the induction of persistent behavioral changes caused by repeated drug exposure. Regardless of the degree of overlap between D1R and D2R, our data point to the importance of cells that express both dopamine and glutamate receptors in the development of locomotor sensitization to cocaine and cocaine-induced place preference.

Using the D1R-NR1^m mice, we have demonstrated that decreased signaling via NMDA receptors in D1R-containing cells is sufficient to impede the development of behavioral sensitization to cocaine. In addition, such a mutation is sufficient to cause a decreased conditioned place preference to cocaine. Because the D1R-NR1^m mice have normal acute response to both amphet-

amine and cocaine, they provide a new model for studying drug addiction. Work on the role of glutamate in addictive behaviors has indicated changes in presynaptic glutamate release (Pierce et al., 1996; Reid et al., 1997), postsynaptic glutamate receptor expression (Fitzgerald et al., 1996; Lu et al., 2003), and changes in the cysteine–glutamate exchanger (Baker et al., 2003). Looking for such changes in the D1R-NR1^{m} mice could contribute to our understanding of which of these changes is necessary for the development of behavioral sensitization to psychostimulants and which are the consequence of the development of behavioral sensitization.

References

Aizman O, Brismar H, Uhlen P, Zettergren E, Levey AI, Forssberg H, Greengard P, Aperia A (2000) Anatomical and physiological evidence for D1 and D2 dopamine receptor colocalization in neostriatal neurons. Nat Neurosci 3:226–230.

Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, Kalivas PW (2003) Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat Neurosci 6:743–749.

Clarke PB, Jakubovic A, Fibiger HC (1988) Anatomical analysis of the involvement of mesolimbocortical dopamine in the locomotor stimulant actions of D-amphetamine and apomorphine. Psychopharmacology (Berl) 96:511–520.

Delfs JM, Schreiber L, Kelley AE (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J Neurosci 10:303–310.

Dudman JT, Eaton ME, Rajadhyaksha A, Macias W, Taher M, Barczak A, Kameyama K, Huganir R, Konradi C (2003) Dopamine D1 receptors mediate CREB phosphorylation via phosphorylation of the NMDA receptor at Ser897-NR1. J Neurochem 87:922–934.

Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ (1996) Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. J Neurosci 16:274–282.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma Jr FJ, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429–1432.

Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 87:6912–6916.

Haracz JL, Belanger SA, MacDonall JS, Sircar R (1995) Antagonist of *N*-methyl-D-aspartate receptors partially prevent the development of cocaine sensitization. Life Sci 57:2347–2357.

Harris GC, Aston-Jones G (2003) Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. Neuropsychopharmacology 28:73–76.

Hotsenpiller G, Wolf ME (2003) Baclofen attenuates conditioned locomotion to cues associated with cocaine administration and stabilizes extracellular glutamate levels in rat nucleus accumbens. Neuroscience 118:123–134.

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci 2:695–703.

Kalivas PW, Alesdatter JE (1993) Involvement of *N*-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. J Pharmacol Exp Ther 267:486–495.

Karler R, Calder LD, Chaudhry IA, Turkanis SA (1989) Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. Life Sci 45:599–606.

Karler R, Chaudhry IA, Calder LD, Turkanis SA (1990) Amphetamine behavioral sensitization and the excitatory amino acids. Brain Res 537:76–82.

Karler R, Calder LD, Turkanis SA (1991) DNQX blockade of amphetamine behavioral sensitization. Brain Res 552:295–300.

Karler R, Calder LD, Bedingfield JB (1994) Cocaine behavioral sensitization and the excitatory amino acids. Psychopharmacology (Berl) 115:305–310.

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507–522.

Kelz MB, Nestler EJ (2000) deltaFosB: a molecular switch underlying long-term neural plasticity. Curr Opin Neurol 13:715–720.

Khan MA, Shoaib M (1996) Neuroanatomical localization of the effects of (+)-HA966 on locomotor activity after cocaine injections to the nucleus accumbens of rats. Brain Res 719:198–202.

Kim HS, Park WK, Jang CG, Oh S (1996) Inhibition by MK-801 of cocaine-induced sensitization, conditioned place preference, and dopamine-receptor supersensitivity in mice. Brain Res Bull 40:201–207.

Konradi C, Cole RL, Heckers S, Hyman SE (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. J Neurosci 14:5623–5634.

Konradi C, Leveque JC, Hyman SE (1996) Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. J Neurosci 16:4231–4239.

Kozell B, Meshul K (2003) Alterations in nerve terminal glutamate immunoreactivity in the nucleus accumbens and ventral tegmental area following single and repeated doses of cocaine. Psychopharmacology (Berl) 165:337–345.

Licata SC, Schmidt HD, Pierce RC (2004) Suppressing calcium/calmodulin-dependent protein kinase II activity in the ventral tegmental area enhances the acute behavioural response to cocaine but attenuates the initiation of cocaine-induced behavioural sensitization in rats. Eur J Neurosci 19:405–414.

Lu L, Grimm JW, Shaham Y, Hope BT (2003) Molecular neuroadaptations in the accumbens and ventral tegmental area during the first 90 days of forced abstinence from cocaine self-administration in rats. J Neurochem 85:1604–1613.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531–3537.

Miner LL, Drago J, Chamberlain PM, Donovan D, Uhl GR (1995) Retained cocaine conditioned place preference in D1 receptor deficient mice. NeuroReport 6:2314–2316.

Moghaddam B, Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science 281:1349–1352.

Mohn AR, Gainetdinov RR, Caron MG, Koller BH (1999) Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell 98:427–436.

Moratalla R, Elibol B, Vallejo M, Graybiel AM (1996) Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. Neuron 17:147–156.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128.

Nestler EJ, Barrot M, Self DW (2001) DeltaFosB: a sustained molecular switch for addiction. Proc Natl Acad Sci USA 98:11042–11046.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 16:1550–1560.

Pitts DK, Marwah J (1987) Neuropharmacology of cocaine: role of monoaminergic systems. Monogr Neural Sci 13:34–54.

Post RM, Weiss SR, Pert A, Fontana D (1992) Conditioned components of cocaine sensitization. Clin Neuropharmacol 15 [Suppl 1]:650A–651A.

Rameau GA, Akaneya Y, Chiu L, Ziff EB (2000) Role of NMDA receptor functional domains in excitatory cell death. Neuropharmacology 39:2255–2266.

Reid MS, Hsu Jr K, Berger SP (1997) Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse 27:95–105.

Reith ME, Meisler BE, Sershen H, Lajtha A (1986) Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem Pharmacol 35:1123–1129.

Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ (1987) Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396:157–198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247–291.

Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598–1604.

Segal DS, Weinberger SB, Cahill J, McCunney SJ (1980) Multiple daily amphetamine administration: behavioral and neurochemical alterations. Science 207:905–907.

Spyraki C, Fibiger HC, Phillips AG (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Res 253:195–203.

Stewart J, Druhan JP (1993) Development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the non-competitive NMDA receptor antagonist, MK-801. Psychopharmacology (Berl) 110:125–132.

Surmeier DJ, Eberwine J, Wilson CJ, Cao Y, Stefani A, Kitai ST (1992) Dopamine receptor subtypes colocalize in rat striatonigral neurons. Proc Natl Acad Sci USA 89:10178–10182.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 56:613–672.

Wolf ME, Jeziorski M (1993) Coadministration of MK-801 with amphetamine, cocaine or morphine prevents rather than transiently masks the development of behavioral sensitization. Brain Res 613:291–294.

Zhang J, Walsh RR, Xu M (2000) Probing the role of the dopamine D1 receptor in psychostimulant addiction. Ann NY Acad Sci 914:13–21.