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J Neurosci. Author manuscript; available in PMC 2013 November 15.

Published in final edited form as:
J Neurosci. 2013 May 15; 33(20): 8640–8649. doi:10.1523/JNEUROSCI.5532-12.2013.

Genetic reconstruction of dopamine D1 receptor signaling in the nucleus accumbens facilitates natural and drug reward responses

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Abstract

The dopamine D1 receptor (D1R) facilitates reward acquisition, and its alteration leads to profound learning deficits. However, its minimal functional circuit requirement is unknown. Using conditional reconstruction of functional D1R signaling in D1R knockout mice, we define distinct requirements of D1R in subregions of the nucleus accumbens (NAc) for specific dimensions of reward. We demonstrate D1R expression selectively in the core region of the NAc (NAc^{Core}), but not the shell (NAc^{Shell}), enhances a unique form of Pavlovian conditioned approach and mediates D1R-dependent cocaine sensitization. However, D1R expression in either the NAc^{Core} or the NAc^{Shell} improves instrumental responding for reward. In contrast, neither NAc^{Core} nor NAc^{Shell} D1R is sufficient to promote motivation to work for reward in a progressive ratio task or for motor learning. These results highlight dissociated circuit requirements of D1R for dopamine-dependent behaviors.

Introduction

Differential gene expression within discrete brain regions expands neural coding capacity and diversifies circuit function. This is exemplified in the striatum where two parallel circuits, the direct and indirect pathway, oppositely regulate thalamocortical loops. These pathways possess a similar neuronal cell type, the medium spiny neuron, yet differ dramatically in connectivity, neuropeptide expression, and genetic profiles. The balance of circuit activation between the direct and indirect pathway is necessary for numerous behaviors including reward processing (Lobo et al., 2010, Beutler et al., 2011). D1R, encoded by the *Drd1a* gene, is highly enriched in the direct pathway (Fig. 1*A,B*) where it facilitates numerous dopamine-dependent functions including appetitive behaviors. Global loss of D1R demonstrates its importance from feeding and reward acquisition to the general ability to thrive (Drago et al., 1994, Xu et al., 1994, Wall et al., 2011). A major unresolved question regarding genes with pleiotropic functions, such as *Drd1a*, is whether a minimal circuit requirement exists for specific behaviors.

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Author Contributions
BG and LZ designed experiments. LZ designed and generated viral constructs. BG performed all experiments. BG and LZ wrote the manuscript.

Conflicts of Interest
The authors declare no conflicts of interests.

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Both genetic and pharmacological studies have investigated the necessity of D1R signaling in different brain regions for acquisition of rewards (Yin et al., 2008, Wall et al., 2011, Salamone et al., 2012). Genetic D1R inactivation in mice demonstrates that despite hyperactivity, these animals show poor motivation to perform instrumental tasks and lack basic Pavlovian learning, which illustrates D1R signaling is necessary somewhere within the brain (Wall et al., 2011). Pharmacological studies narrowed the potential candidate brain regions necessary for reward processing. Infusion of D1R antagonists into either the prefrontal cortex (Baldwin et al., 2002), dorsal striatum (Lovinger, 2010), amygdala (Berglind et al., 2006, Tye et al., 2010), or NAc (Smith-Roe and Kelley, 2000) each individually disrupts certain aspects of reward. However, whether any of these brain regions is minimally required for different dimensions of reward is unresolved.

One caveat to locally infusing antagonists to establish regional importance of receptor function is the potential to inactivate both postsynaptic and presynaptic receptors. In comparison, conditional gene inactivation provides cell selectivity, but does not typically permit regional selectivity, nor does it exclude necessary roles for the gene in other cells/ regions. Alternatively, knockout mice are operationally a blank slate for a specific gene of interest, so conditional, anatomically restricted restoration to neurons endogenously expressing the gene can test its minimal circuit requirement. Here we developed a model system for global D1R inactivation and cell-selective, regional restoration to investigate whether D1R activation in either the NAc^{Core} or the NAc^{Shell} is the minimal requisite to mediate distinct aspects of reward. We find exclusive expression of D1R in the NAc^{Core} of D1R knockout mice promoted Pavlovian conditioned approach and facilitated behavioral sensitization to repeated cocaine administration, thus highlighting the essential role of this brain region for both natural and drug rewards. In contrast, NAc^{Shell} D1R expression did not alter Pavlovian conditioned approach, nor did it restore cocaine sensitization. However, either NAc^{Core} or NAc^{Shell} D1R expression stimulated instrumental responding for reward, but neither improved motivation to work for reward or motor learning.

Materials and Methods

Mice

Generation of mice with inactivation of *Drd1a* by insertion of Cre recombinase are described elsewhere (Heusner et al., 2008). $Drd1a^{Cre/Cre}$ mice were generated by crossing heterozygous $Drd1a^{Cre/+}$ mice and were born at the expected Mendelian ratio. An approximately equal number of male and female mice were used for all experiments. All experimental protocols were approved by the University of Washington Institutional Animal Care and Use Committee. Mice were housed on a 12:12 light:dark cycle and given ad libitum food and water except during food restriction to 85% of their ad libitum bodyweight.

Generation of AAV-FLEX-D1RGFP, viral injections, and experimental groups

The AAV-FLEX-D1RGFP was generated by PCR amplification of D1R from genomic DNA (C57Bl6/J) using primers, 5'-GATATCACCGGTATGGCTCCTAACACTTCTAC-3' and 5'-GATATCGCGGCCGCGGTTGAATGCTGTCCGCTGT-3'. The 1.3kb PCR product was subcloned into AM/CBA-FLEX-EGFP-WPRE-bGH in frame with eGFP. AAV was generated as previously described (Zweifel et al., 2008). For stereotaxic viral injections, 0.5μl of AAV-FLEX-D1RGFP (titer $\sim 1\times10^{12}$/ml) or control AAV-FLEX-GFP (titer $\sim 1\times10^{12}$/ml) was bilaterally injected into the NAc^{Core} (x =+/-1.0, y=+1.3*F, z=-4.25) or NAc^{Shell} (x=+/-0.4, y=+1.3*F, z=-5.0), F=(Lambda minus Bregma)/4.21. To control for effects of site-specific injections and viral-mediated D1R expression in restricted NAc subregions, we generated the following experimental groups: NAc^{Core}, Het GFP-NAc^{Core} ($Drd1a^{Cre/+}$; AAV-FLEX-GFP, NAc^{Core} injected); Het D1R-NAc^{Core} ($Drd1a^{Cre/+}$; AAV-

FLEX-D1RGFP, NAc^{Core} injected); Mutant GFP-NAc^{Core} (*Drd1a*$^{Cre/Cre}$; AAV-FLEX-GFP, NAc^{Core} injected); Mutant D1R-NAc^{Core} (*Drd1a*$^{Cre/Cre}$; AAV-FLEX-D1RGFP, NAc^{Core} injected). NAc^{Shell}, Het GFP-NAc^{Shell} (*Drd1a*$^{Cre/+}$; AAV-FLEX-GFP, NAc^{Shell} injected); Het D1R-NAc^{Shell} (*Drd1a*$^{Cre/+}$; AAV-FLEX-D1RGFP, NAc^{Shell} injected); Mutant GFP-NAc^{Shell} (*Drd1a*$^{Cre/Cre}$; AAV-FLEX-GFP, NAc^{Shell} injected); Mutant D1R-NAc^{Shell} (*Drd1a*$^{Cre/Cre}$; AAV-FLEX-D1RGFP, NAc^{Shell} injected). D1R-NAc^{Core} and D1R-NAc^{Shell} mice were compared to their respective heterozygous and mutant control groups. Following surgery, mice recovered for two weeks before behavioral testing. Viral expression was confirmed with immunohistochemistry with the D1R antibody, or with the GFP antibody that detected either GFP or D1RGFP.

Pavlovian Conditioning

Training was performed in operant chambers (Med Associates) as previously described (Parker et al., 2010). Briefly, animals received daily Pavlovian training for seven days that included 25 trials per session. During each trial, two levers were presented for 10s, which co-terminated with a 20mg food pellet delivered non-contingently (Bio-serve). Video tracking was performed with Ethovision (Noldus) on the final day to score lever or food receptacle contacts.

Instrumental Conditioning

Four days of instrumental conditioning were performed with 50 trials per session where a single lever press delivered a single food reward pellet. Food receptacle head entries were required to start the next trial. The session continued until 50 trials were completed or two hours had elapsed. For progressive ratio testing, one reward pellet was delivered per completed trial where the lever press requirement increased with a nonarithmetic schedule (1,1,4,7,13,19,25,34,43,52,61,73...). The breakpoint was the last completed trial before 3 minutes of lever pressing inactivity or a total four hour session time-out.

Rotarod

Motor learning was measured on a rotarod (4 to 40 RPM over two minutes) with three trials per day for five days (Columbus Instruments).

Pharmacology studies in locomotion chambers

For D1R agonist studies, SKF-81297 was administered i.p. (intraperitoneal) at 7.5 mg/kg. Locomotor activity was measured for 90 minutes in locomotion chambers (Opto-M3; Columbus Instruments). For cocaine sensitization studies, baseline locomotion recordings were measured for 90 minutes. For two days, animals received injections of 0.9% saline, which were averaged. For the next five days, cocaine was administered s.c. (subcutaneous) at 20 mg/kg and locomotor activity measured for 90 minutes.

Immunohistochemistry

For measuring c-Fos expression, 90 minutes prior to euthanasia and 4% paraformaldehyde perfusion, animals received either 0.9% saline or 7.5 mg/kg of SKF-81297. 30 μm frozen sections were collected between +1.1 to +1.5 (relative to bregma, A-P axis) and stained with primary antibody: GFP, mouse monoclonal , 1:1000 (Invitrogen); c-Fos, rabbit polyclonal, 1:1000 (Calbiochem); D1R, rat monoclonal, 1:500 (Sigma-Aldrich); all secondary antibodies, 1:200 (Jackson ImmunoResearch). For c-Fos quantification, equal camera exposures were taken and c-Fos positive cells were counted with ImageJ (NIH) in three sections per animal at a defined ROI ($350 \times 500\mu m$ box centered on either the anterior commissure for the NAc^{Core} injected groups or in the ventral medial portion of the NAc^{Shell} for the NAc^{Shell} injected groups). To measure the pattern of viral expression for D1R-

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NAc^{Core} and D1R-NAc^{Shell} mice, Illustrator (Adobe) was used to trace the bilateral viral expression at the section closest to +1.3 (relative to bregma, A-P axis).

Statistical Analyses

Data was analyzed using Excel (Microsoft) and Matlab (Mathworks). Additional statistical calculations were performed in Prism (GraphPad). All data was analyzed by two-way repeated measures ANOVA or one-way ANOVA as indicated.

Results

Functional restoration of D1R signaling in the NAc

To establish our model system, we exclusively expressed D1R in either the NAc^{Core} or the NAc^{Shell} utilizing a mouse line where D1R expression was functionally inactivated by inserting a *Cre* recombinase expression cassette into the open reading frame of the *Drd1a* locus (Heusner et al., 2008). This results in selective expression of *Cre* in D1R containing cells. Mice homozygous for the *Cre* insertion are null mutants, *Drd1a$^{Cre/Cre}$* (D1R mutants), and do not have detectable D1R protein levels (Fig. 1*C,D*). Similar to previously published D1R knockout lines, D1R mutants generated by *Cre* insertion are indistinguishable from other D1R knockout mouse lines (Drago et al., 1994, Xu et al., 1994). To re-express D1R in an anatomically restricted manner, we generated an adeno-associated viral vector containing a Cre-conditional D1R-GFP expression cassette (AAV-FLEX-D1RGFP, Fig. 1*A*). D1R expression restricted to either the NAc^{Core} (D1R-NAc^{Core}) or the NAc^{Shell} (D1R-NAc^{Shell}) was achieved by bilateral stereotaxic injection of AAV-FLEX-D1RGFP into D1R mutants (Fig. 1*C-F*).

We next validated functional restoration of D1R in the NAc. D1R activation stimulates locomotor activity, and dopamine signaling exclusively in the NAc facilitates locomotor activation (Swanson et al., 1997, Heusner et al., 2003). Therefore, to confirm D1R activation in D1R-NAc^{Core} and D1R-NAc^{Shell} mice, we measured locomotor responses to systemic administration of the D1R agonist SKF-81297 (7.5 mg/kg). In the NAc^{Core} groups, GFP-NAc^{Core} mutant mice (n=7) displayed little locomotor response to the drug (Fig. 2*A*). In contrast, D1R-NAc^{Core} mice (n=7) showed a strong agonist effect, which was indistinguishable from heterozygous control groups (Het GFP-NAc^{Core}, n=7; or Het D1R-NAc^{Core}, n=8; two-way repeated measures ANOVA, genotype × time, $F_{(63,525)}=1.4$, $p=0.0282$; Fig. 2*A*). D1R-NAc^{Shell} (n=9) mice also responded to SKF-81297 with significantly increased locomotor activity compared to GFP-NAc^{Shell} mutants (n=7; two-way repeated measures ANOVA, genotype × time, $F_{(63,777)}=3.5$, $p<0.0001$; Fig. 2*B*), but did not respond as strongly as heterozygous control mice (Het GFP-NAc^{Shell}, n=12; or Het D1R-NAc^{Shell}, n=13; Fig.2*B*).

To further confirm signaling events downstream of D1R activation are present in D1R-NAc^{Core} and D1R-NAc^{Shell} mice, we quantified c-Fos expression around the area of viral restoration following SKF-81297 administration (7.5 mg/kg; Fig. 2*C-F*). D1R-NAc^{Core} (n=6) and control mice (Het GFP-NAc^{Core}, n=5; or Het D1R-NAc^{Core}, n=5) showed robust c-Fos induction (one-way ANOVA, $F_{(4,25)}=12.2$, $p<0.0001$; Fig. 2*C*). In contrast, saline injected controls (All genotypes, n=8) and SKF-81297 treated GFP-NAc^{Core} mutants (n=6) showed negligible c-Fos expression (Fig. 2*E*). Similarly, D1R-NAc^{Shell} (n=7) and control mice (Het GFP-NAc^{Shell}, n=10; or Het D1R-NAc^{Shell}, n=10) also displayed strong induction of c-Fos compared to saline injected controls (All genotypes, n=9) and SKF-81297 treated GFP-NAc^{Shell} mutants (n=6; one-way ANOVA, $F_{(4,37)}=32.90$, $p<0.0001$; Fig. 2*D,F*). Therefore, re-expression of D1R to either the NAc^{Core} or the NAc^{Shell} can restore signaling and behavioral responsiveness to D1R agonist.

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Unique role of D1R in the NAc for Pavlovian conditioning

Having established the ability to regionally restrict expression of D1R to either the NAc^{Core} or the NAc^{Shell}, we next examined D1R in these regions for reward processing. In Pavlovian conditioning, animals learn to associate a predictive cue with a reward outcome. These dopamine-dependent associations manifest behaviorally as conditioned approach to either the reward (goal-tracking) or the predictive conditioned stimulus (CS) cue (sign-tracking) (Flagel et al., 2011). To determine if D1R in the NAc is sufficient for Pavlovian conditioning, we trained mice to associate a reward-predictive cue (10 sec lever extension) with food pellet delivery. In mice, conditioned approach typically manifests as goal-tracking (Parker et al., 2010) measured by calculating the difference between the head entry rate during the CS presentation and the inter-trial interval. In contrast to heterozygous control mice in the NAc^{Core} group (Het GFP-NAc^{Core}, n=8; or Het D1R-NAc^{Core}, n=8), we did not find the head entry rate in D1R-NAc^{Core} mice (n=7) was significantly above their respective mutant control group (GFP-NAc^{Core}, n=7) during CS presentation (two-way repeated measures ANOVA, genotype × time, $F_{(18,156)}$=1.87, p=0.0222; Fig. 3*A*). Similarly, D1R-NAc^{Shell} mice (n=9) also failed to increase their head entry rate significantly above respective mutant controls (GFP-NAc^{Shell}, n=7; Fig. 3*B*). Intriguingly, while viewing the animals during conditioning, we observed D1R-NAc^{Core} mice (Fig. 3*C*), but not D1R-NAc^{Shell} mice (Fig. 3*D*), exhibited a heightened approach behavior where they repeatedly shuttled between the food receptacle and levers, a behavior undetected by strictly measuring head entries. To quantify this behavior, on the final day of conditioning, we video recorded each trial (25 total) and scored conditioned approach to the food receptacle, lever, or both (Fig. 3*E,F*). We found GFP-NAc^{Core} and GFP-NAc^{Shell} mutant mice made significantly fewer conditioned approaches compared to their respective heterozygous controls (Fig. 3*E,F*). However, D1R-NAc^{Core} mice exhibited conditioned approach to either the food receptacle, lever, or both (one-way ANOVA, $F_{(3,26)}$=19.68 , p<0.0001; Fig 3*E*). In very few trials did control or D1R-NAc^{Core} mice solely approach the lever, indicating they are not exclusively sign-tracking, but performing a hybrid goal-tracking/sign tracking behavior. D1R-NAc^{Shell} mice did not display this behavior (one-way ANOVA, $F_{(3,38)}$=113.1, p<0.0001; Fig 3*F*).

Sufficiency of D1R in the NAc for instrumental conditioning

Conditioned approach to the lever by D1R-NAc^{Core} mice during Pavlovian conditioning suggests these animals have assigned some value to the cue, so we asked whether they would perform an instrumental response (lever press) to acquire reward. Immediately following Pavlovian conditioning, mice were given a simple fixed ratio (FR) schedule of one lever press for one reward pellet (FR1). As reported previously (El-Ghundi et al., 2003, Caine et al., 2007, Wall et al., 2011), D1R null mice (GFP-NAc^{Core} mutant, n=7) were severely deficient in this task relative to heterozygous controls (Het GFP-NAc^{Core} n=8; or Het D1R-NAc^{Core}, n=8; Fig. 4*A*). Remarkably, performance of D1R-NAc^{Core} mice (n=7) was significantly more robust than GFP-NAc^{Core} mutants (n=7; two-way repeated measures ANOVA, genotype × time, $F_{(9,78)}$=4.11, p=0.0002; Fig. 4*A*). Surprisingly, despite previously displaying no Pavlovian conditioned approach behavior to the levers, D1R-NAc^{Shell} mice (n=9) also displayed significantly increased instrumental responding relative to their respective mutant controls (GFP-NAc^{Shell} mutant, n=7; two-way repeated measures ANOVA, effect of genotype, $F_{(3,38)}$=14.20, p<0.0001; effect of time, $F_{(3,114)}$=7.24, p=0.0002; Fig. 4*B*). Furthermore, cumulative reward acquisition in both D1R-NAc^{Core} and D1R-NAc^{Shell} mice revealed both groups completed or nearly completed all lever presses (NAc^{Core} experiment: two-way repeated measures ANOVA, genotype × time, $F_{(720,6240)}$=4.33, p<0.0001; Fig. 4*C*; NAc^{Shell} experiment: two-way repeated measures ANOVA, genotype × time, $F_{(720,9120)}$=8.97, p<0.0001; Fig. 4*D*).

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The improved performance of D1R-NAc^{Core} and D1R-NAc^{Shell} mice in instrumental behavior compared to mutant mice suggests these animals are capable of performing an action required to attain reward. To explore whether their enhanced instrumental performance reflects increased incentive to perform work, we tested mice in a progressive ratio task, which measured the animal's breakpoint to an escalating increase in lever presses required to deliver a single reward pellet. Both D1R-NAc^{Core} and D1R-NAc^{Shell} mice showed a marginal yet statistically insignificant increase in breakpoint compared to D1R mutants (Fig. 4*E,F*). However, D1R-NAc^{Core}, D1R-NAc^{Shell}, and mutant control breakpoints were significantly smaller in comparison to heterozygous control mice (NAc^{Core} experiment: one-way ANOVA, $F_{(3,12)}$=7.029, p=0.0055; Fig. 4*E*; NAc^{Shell} experiment: one-way ANOVA, $F_{(3,38)}$=16.96, p<0.0001; Fig.4*F*). Therefore, although D1R-NAc^{Core} and D1R-NAc^{Shell} mice can perform a simple fixed ratio task (FR1), when challenged with escalating costs to obtain reward, they fail to perform at the level of controls.

To test whether instrumental performance by D1R-NAc^{Core} and D1R-NAc^{Shell} mice simply reflects improved motor coordination, we assayed mice in a rotarod task. Similar to mutants (GFP-NAc^{Core}, n=7; or GFP-NAc^{Shell}, n=7), both D1R-NAc^{Core} (n=7) and D1R-NAc^{Shell} (n=9) mice failed to improve over five days of training and performed significantly worse than their respective heterozygous controls (NAc^{Core} experiment: Het GFP-NAc^{Core}, n=8; or Het D1R-NAc^{Core}, n=8; two-way repeated measures ANOVA, genotype × time, $F_{(12,104)}$=2.47, p=0.0071; Fig. 5*A*; NAc^{Shell} experiment: Het GFP-NAc^{Shell}, n=13; or Het D1R-NAc^{Shell}, n=13; two-way repeated measures ANOVA, genotype × time, $F_{(12,152)}$=3.70, p<0.0001; Fig. 5*B*). Thus, D1R in either the NAc^{Core} or the NAc^{Shell} can facilitate instrumental performance despite the inability to improve motor coordination, indicating a dissociable minimal requirement of D1R for these behaviors.

Differential minimal requirement of D1R in the NAc^{Core} and NAc^{Shell} for cocaine sensitization

The ability to assign value to cues or actions requires neuroplastic changes in the NAc that depends upon D1R signaling (Kelley, 2004). Drugs of abuse usurp this endogenous reward system leading to escalated incentive value for the drug, which can be observed as psychomotor sensitization to repeated drug administration (Robinson and Berridge, 2008). To explore whether D1R in either the NAc^{Core} or the NAc^{Shell} is sufficient to mediate behavioral adaptation to elevated synaptic dopamine levels associated with repeated drug exposure, we measured locomotor sensitization in response to daily cocaine injections (Fig. 6*A-D*). Locomotor activity was monitored for 90 minutes prior to cocaine administration (20 mg/kg) and then for an additional 90 minutes (Fig. 6*A,B*). After five days of cocaine injections in the NAc^{Core} mice, both D1R-NAc^{Core} (n=7) and heterozygous control mice (Het GFP-NAc^{Core}, n=7; or Het D1R-NAc^{Core}, n=8) robustly sensitized, which was not observed in mutant control mice (GFP-NAc^{Core} mutant, n=7; two-way repeated measures ANOVA, genotype × time, $F_{(105,875)}$=4.10, p<0.0001; Fig. 6*C*). In contrast to D1R-NAc^{Core} mice, D1R-NAc^{Shell} animals (n=9) failed to show acute responses or locomotor sensitization to cocaine; however, their respective heterozygous controls showed robust sensitization (Het GFP-NAc^{Shell}, n=12; or Het D1R-NAc^{Shell}, n=13; two-way repeated measures ANOVA, genotype × time, $F_{(105,1295)}$=9.40, p<0.0001; Fig. 6*D*). Intriguingly, D1R-NAc^{Core}, D1R-NAc^{Shell} and mutant controls showed hyper-novelty responses during the first 90 minutes of habituation to the locomotion chambers, indicating neither D1R in the NAc^{Core} nor the NAc^{Shell} is sufficient to reverse this behavioral phenotype (Fig. 6*A-D*). To account for this hyperactivity, we normalized cumulative cocaine responses by subtracting the first 90 minutes from the last 90 minutes of activity, which further highlighted locomotor sensitization to cocaine in D1R-NAc^{Core}, but not D1R-NAc^{Shell} mice (NAc^{Core} experiment: two-way repeated measures ANOVA, genotype × time, $F_{(15,125)}$=2.67, p=0.0015; Fig.6*E*;

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NAc^{Shell} experiment: two-way repeated measures ANOVA, genotype × time, $F_{(15,185)}$=2.09, p=0.0121; Fig. 6*F*).

Discussion

Defining the minimal requirements for genes expressed within a neural circuit is essential to understand how circuits regulate different dimensions of behavior. Studying minimal gene requirements within a specific circuit node can be achieved using different strategies. For example, non-conditional viral vectors can be injected into a region of interest in a conventional global knockout (Carlezon et al., 1997), but this yields ectopic expression in cells that do not endogenously express the gene. In contrast, select promoters can drive more specific expression from a viral vector (Ferguson et al., 2011), but frequently the minimal promoter is too large for efficient viral packaging. Alternatively, utilizing the approach described here, the endogenous gene locus drives *Cre* expression and simultaneously creates a global knockout. Thus, conditional viral vectors can be introduced into select regions to re-express the gene only in cells endogenously expressing the gene. Numerous *Cre*-knockin lines have been generated, so this approach will prove broadly useful for studying minimal gene requirements.

Utilizing this strategy, we show functional D1R signaling can be regenerated in an anatomically restricted and cell-selective manner. This is illustrated by restoring locomotor activation and the induction of c-Fos in response to the D1R agonist SKF-81297. Although these results are consistent with functional re-expression of D1R, without ultrastructural analysis, we cannot unequivocally establish the protein is precisely targeted to the endogenous location within the cell or that levels are not excessive. However, immunolocalization of recombinant D1R in both D1R-NAc^{Core} and D1R-NAc^{Shell} mice demonstrate the protein is largely localized to neural processes, remarkably similar to staining of the endogenous protein in heterozygous control mice. In addition, AAV-FLEX-D1RGFP delivered to the NAc^{Core} or the NAc^{Shell} of heterozygous mice does not significantly alter behavior indicating expression does not perturb endogenous receptor function. Although D1RGFP in the NAc^{Core} of heterozygous mice shifted these animals towards more hybrid tracking behavior during Pavlovian conditioning, it was not statistically significant; this is discussed further below.

Systemic administration of D1R agonist induced locomotion in both D1R-NAc^{Core} and D1R-NAc^{Shell} mice. These results are consistent with a previously published report showing restoration of dopamine to the NAc^{Core} in dopamine-deficient animals is sufficient to promote psychomotor activation by amphetamine, an effect blocked by D1- and D2-type receptor antagonist (Heusner et al., 2003). Furthermore, infusing dopamine into either the NAc^{Core} or the NAc^{Shell} potentiates locomotor activity (Swanson et al., 1997), but in contrast to our findings, this study showed infusing D1R agonist had a greater effect in the NAc^{Shell}. Our results demonstrate D1R-dependent behavioral sensitization to cocaine can be mediated exclusively by D1R activation in the NAc^{Core} but not the NAc^{Shell}. Previous studies have reported repeated cocaine administration enhances D1R sensitivity of NAc neurons (White et al., 1993) and increases dopamine release in both the NAc^{Core} and the NAc^{Shell} (Addy et al., 2010). Glutamate plasticity within the NAc^{Core} following repeated cocaine has also been reported and proposed to be dependent on D1R (Pierce et al., 1996). In contrast to our results, repeated cocaine administration has been shown to enhance sensitivity to amphetamine infusion into the NAc^{Shell}, but not the NAc^{Core}. This effect was observed after long-term, but not short-term, withdrawal; however, early acquisition of sensitized responses was not investigated (Pierce and Kalivas, 1995). Temporal differences in electrophysiological (Kourrich and Thomas, 2009) and morphological changes (Dumitriu et al., 2012) have been found between the NAc^{Core} and the NAc^{Shell} after cocaine

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sensitization, suggesting independent functions of these brain regions acting over different time courses for discrete facets of drug-related behavior. Consistent with this, D1R signaling in the NAc^{Core} and the NAc^{Shell} is essential for distinct aspects of drug self-administration (Anderson et al., 2003, Bachtell et al., 2005, Bari and Pierce, 2005, Schmidt et al., 2006, Bossert et al., 2007, Laviolette et al., 2008, Shin et al., 2008, Suto and Wise, 2011), so future experiments with combined viral restoration in both the NAc^{Core} and the NAc^{Shell} during various stages of drug-seeking will help to address this important question. We should also note that D1R antagonists infused into the prefrontal cortex can block cocaine sensitization (Sorg et al., 2001). One potential explanation for the apparent necessity of D1R in one scenario but not another is the D5 receptor, which is also inhibited by D1R antagonist and is highly expressed in the prefrontal cortex (Oda et al., 2010). Consistent with this, locomotor responding to cocaine has been shown to be attenuated dose-dependently in D5R knockout mice (Elliot et al., 2003); however, others have not reported similar findings (Karlsson et al., 2008). Finally, although it is possible developmental compensatory changes occur in D1R mutants allowing a smaller subset of D1R-expressing brain regions to be minimally sufficient, this is unlikely since re-expression of D1R in the NAc^{Shell} did not facilitate all behaviors restored by D1R in the NAc^{Core}.

Restricted expression of D1R in the NAc^{Core} reveals additional insight into the circuit level requirement for D1R during appetitive Pavlovian conditioning. The NAc^{Core} is conventionally associated with preparatory Pavlovian conditioned approach (Flagel et al., 2011), CS-US associations during conditioned reinforcement (Parkinson et al., 1999), and the generalized form of Pavlovian-Instrumental transfer (Corbit and Balleine, 2011). We were initially surprised D1R-NAc^{Core} animals failed to demonstrate “normal” conditioned approach behavior typically observed in mice. Instead, we observed a hybrid goal/sign tracking conditioned approach. Mice almost exclusively exhibit goal-tracking conditioned approach behavior, as evidenced by Het GFP-NAc^{Core} mice that predominantly approached the food receptacle exclusively. In contrast to mice, rats show individual preference to either goal or sign-track, but importantly, only sign-tracking but not goal-tracking is sensitive to the broad spectrum dopamine receptor antagonist flupenthixol, and sign-tracking rats have higher levels of D1R in the NAc (Flagel et al., 2007). Notably, we find exclusive expression of D1R in the NAc^{Core} promotes the highest degree of conditioned approaches to the lever, with Het D1R-NAc^{Core} mice displaying a more intermediate level of hybrid tracking. These results indicate restoring D1R selectively to the NAc^{Core} potentially overrides an innate goal-tracking preference in mice. Additionally, sign-tracking in rats is associated with enhanced sensitization to drugs of abuse (Flagel et al., 2010). In accordance, we find D1R-NAc^{Core} mice have the highest levels of sensitization, further supporting the link between NAc^{Core} D1R, sign-tracking, and drug sensitization. Altogether, these results suggest shifting the balance of D1R activation in the brain more heavily towards the NAc^{Core}, or exclusively to the NAc^{Core}, shifts behavior towards sign-tracking, a conditioned approach strategy more sensitive to dopamine levels and associated with enhanced behavioral responding to drugs of abuse.

The development of instrumental responding in D1R-NAc^{Core} and D1R-NAc^{Shell} mice is consistent with these animals assigning value to the lever to perform simple action-outcome responses. Although both groups responded significantly better than mutant control mice, they may have done so through different mechanisms. D1R-NAc^{Core} mice demonstrated conditioned approach to the levers, suggesting they assigned incentive salience to the levers, possibly strengthening the action-outcome association required in the subsequent instrumental conditioning sessions. This is consistent with observations that suppression of excitatory inputs to the NAc^{Core} from the amygdala impairs cued reward retrieval, and activation of this projection facilitates instrumental responding, which is blocked by systemic administration of D1R antagonist (Stuber et al., 2011). In contrast, D1R-NAc^{Shell}

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mice demonstrated instrumental performance despite their lack of Pavlovian conditioned approach, suggesting D1R-NAc^{Shell} mice have elevated instrumental responding through a different mechanism compared to D1R-NAc^{Core} mice. This is consistent with these animals having restored consummatory or hedonic processes (Yin et al., 2008) sufficient for action-outcome responding, but lacking the ability to form conditioned reward associations. Intriguingly, although D1R-NAc^{Core} and D1R-NAc^{Shell} mice performed the simple instrumental response, they demonstrated profound motivational deficits to work for reward. These results suggest dissociable circuit requirements for performing tasks when costs are low versus high. One explanation for this finding is lack of D1R expression in other brain regions could make these mice more sensitive to extinction and/or contingency changes during a progressive ratio task. For example, these mice lack D1R in the prefrontal cortex, hippocampus, and amygdala, all structures known to be necessary for cost-benefit decision making (Floresco et al., 2008). In addition, dorsal striatum D1R signaling is necessary for habit formation (Lovinger, 2010), so lacking D1R in the dorsal striatum of D1R-NAc^{Core} or D1R-NAc^{Shell} mice may prevent transforming goal-directed actions into habitual responses.

Failure of D1R-NAc^{Core} and D1R-NAc^{Shell} mice to improve motor coordination and attenuate novelty-induced hyperactivity further highlights the selective nature of D1R function in circuits underlying distinct dopamine-dependent behaviors. Thus, our model allows for the systematic determination of the minimal requirements of D1R signaling in discrete brain regions to establish functional D1R-dependent circuit maps underlying dopamine-dependent behaviors. Establishing functional maps of where gene expression is minimally required to mediate specific functions is essential for therapeutic approaches requiring targeted intervention. Our approach provides a critical first step in establishing a method to define the minimal requirements for D1R in regulating complex behavior. Therefore, future experiments to define the minimal requirements of D1R for other behaviors will be essential for understanding the neural circuitry underlying dopamine-dependent processes and disease.

Acknowledgments

Drd1a^{Cre} mice were generously provided by Dr. Richard Palmiter. We thank Dr. Ali Güler for assistance with locomotion and sensitization experiments. We also thank all members of the Zweifel lab for their support and critical reading of the manuscript. This work was supported by the National Institutes of Health R01MH094536 (LSZ) and T32DA7278 (BBG).

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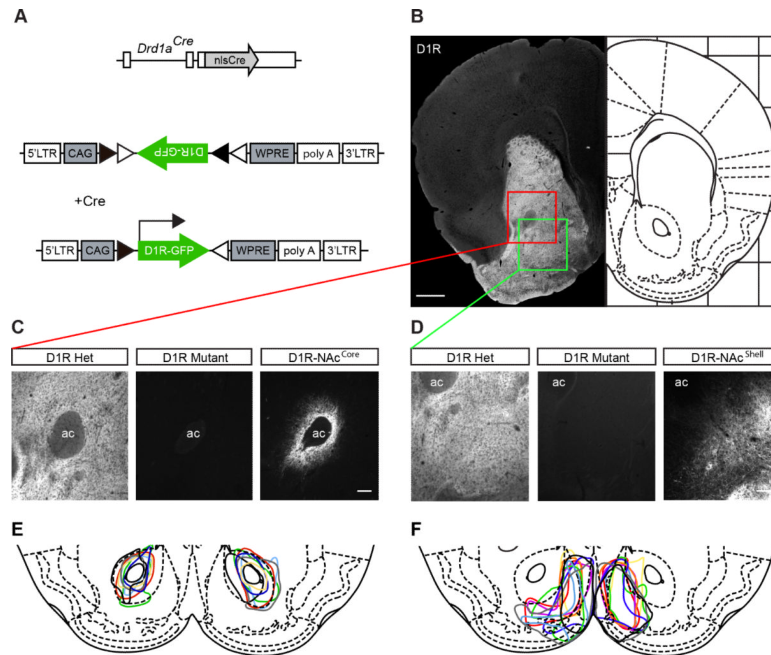

Figure 1.

Conditional viral restoration of D1R expression in either the NAc^{Core} or the NAc^{Shell}. ***A***, Schematic representation of *Drd1a*^{Cre} allele and AAV-FLEX-D1RGFP construct. ***B***, Left: D1R protein expression is highly enriched in the striatum; right: Allen Brain Atlas, Bregma +1.34, (Dong, 2008). ***C***, Higher magnification of NAc^{Core} region from ***B***. D1R is completely absent in D1R mutants, but selectively expressed in the NAc^{Core} with AAV-FLEX-D1RGFP. ***D***, Higher magnification of NAc^{Shell} region from ***B***. D1R is completely absent in D1R mutants, but selectively expressed in the NAc^{Shell} with AAV-FLEX-D1RGFP. ***E,F***, Tracing of bilateral D1RGFP expression in D1R-NAc^{Core} (n=7) and D1R-NAc^{Shell} (n=9) mice. ***B***, Scale bar: 500μm. ***C,D***, Scale bars: 100μm. ac, anterior commissure. Mean ± s.e.m.

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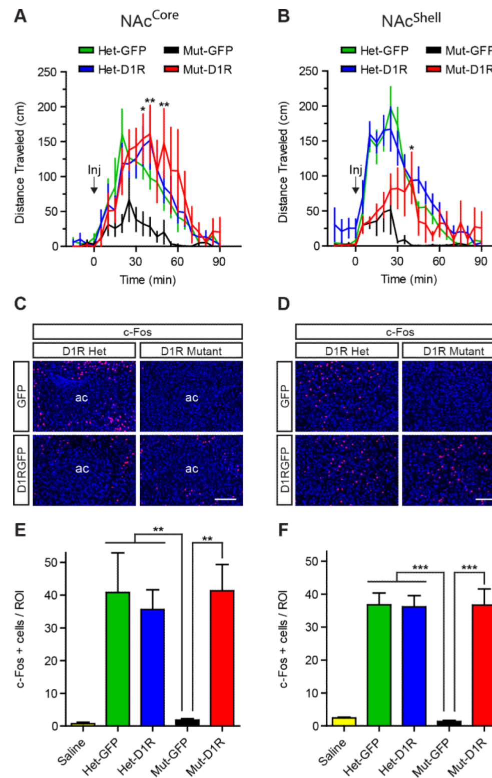

Figure 2.

D1R in either the NAc^{Core} or the NAc^{Shell} restores behavioral responsiveness and functional D1R signaling.

A,B, Locomotor response to D1-agonist, SKF-81297 in NAc^{Core} and NAc^{Shell} mice (NAc^{Core}: Het-GFP, n=7; Het-D1R, n=8; Mut-GFP, n=7; Mut-D1R, n=7; NAc^{Shell}: Het-GFP, n=12; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). ***C,D***, SKF-81297 induced c-Fos expression (red) in D1R-NAc^{Core} (Mut-D1R) and control mice, and D1R-NAc^{Shell} (Mut-D1R) and control mice. Brain sections were counterstained with Hoechst (blue). ***E,F***, Quantification of c-Fos positive cells in NAc^{Core} and NAc^{Shell} mice (NAc^{Core}: Saline controls, all genotypes, n=8; Het-GFP, n=5; Het-D1R, n=5; Mut-GFP, n=6; Mut-D1R, n=6; NAc^{Shell}: Saline controls, all genotypes, n=9; Het-GFP, n=10; Het-D1R, n=10; Mut-GFP, n=6; Mut-D1R, n=7). ***A,B***, Bonferroni's multiple comparison test, *$p<0.05$, **$p<0.01$, D1R-NAc^{Core} or D1R-NAc^{Shell} mice vs D1R mutants. ***E,F***, Tukey's multiple comparison test, **$p<0.01$, ***$p<0.001$, D1R mutants vs all other groups. ***C,D***, Scale bars: 100μm. Mean ± s.e.m.

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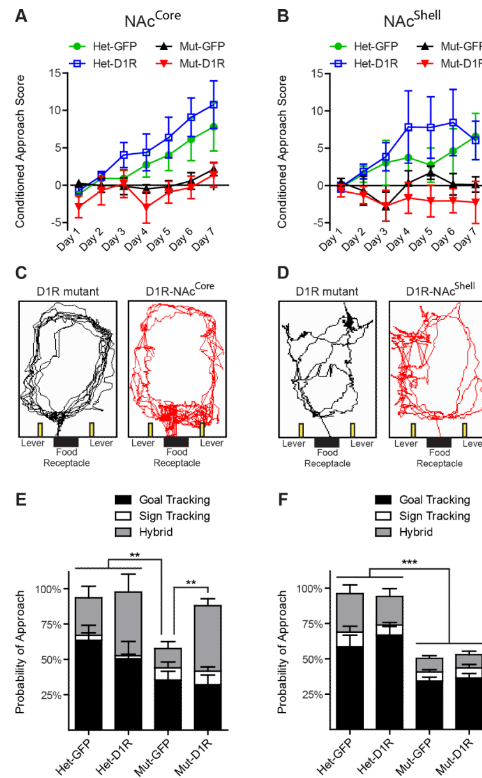

Figure 3.

D1R in the NAc^{Core}, but not the NAc^{Shell}, facilitates Pavlovian conditioned approach behavior.

A,B, Pavlovian conditioned approach score, [(CS head entry rate) – (ITI head entry rate)] for NAc^{Core} and NAc^{Core} mice (NAc^{Core}: Het-GFP, n=8; Het-D1R, n=8; Mut-GFP, n=7; Mut-D1R, n=7; NAc^{Shell}: Het-GFP, n=13; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). ***C,D***, Track tracing from last trial of day 7 for D1R mutant and D1R-NAc^{Core} mice, and D1R mutant and D1R-NAc^{Shell} mice illustrating conditioned approach to the lever and receptacle in D1R-NAc^{Core} mice, but not in mutant control groups or D1R-NAc^{Shell} mice. ***E,F***, Quantification of conditioned approach behavior for NAc^{Core} and NAc^{Shell} mice from ***A,B***. ***E,F***, Tukey's multiple comparison test, **p<0.01, ***p<0.001. Mean ± s.e.m.

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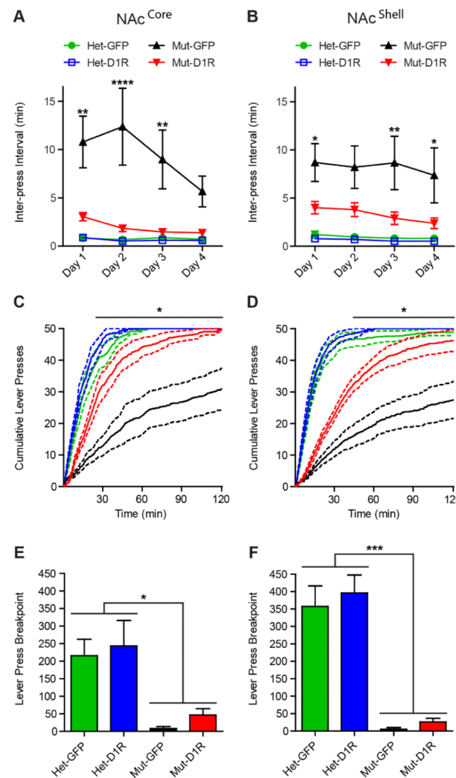

Figure 4.

D1R in either the NAc^{Core} or the NAc^{Shell} promotes instrumental conditioning. ***A,B***, Inter-press interval during instrumental conditioning over four days for NAc^{Core} and NAc^{Shell} mice (NAc^{Core}: Het-GFP, n=8; Het-D1R, n=8; Mut-GFP, n=7; Mut-D1R, n=7; NAc^{Shell}: Het-GFP, n=13; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). ***C,D***, Cumulative lever presses on Day 4 for NAc^{Core} and NAc^{Shell} mice from ***A,B***. ***E,F***, Progressive ratio breakpoint analysis for NAc^{Core} and NAc^{Shell} mice (NAc^{Core}: Het-GFP, n=4; Het-D1R, n=4; Mut-GFP, n=4; Mut-D1R, n=4; NAc^{Shell}: Het-GFP, n=13; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). ***A-D***, Bonferroni's multiple comparison test, *$p<0.05$, **$p<0.01$, ****$p<0.0001$, D1R-NAc^{Core} or D1R-NAc^{Shell} mice vs D1R mutants. ***E,F***, Tukey's multiple comparison test, *$p<0.05$, ***$p<0.001$, D1R mutants vs all other groups. Mean ± s.e.m.

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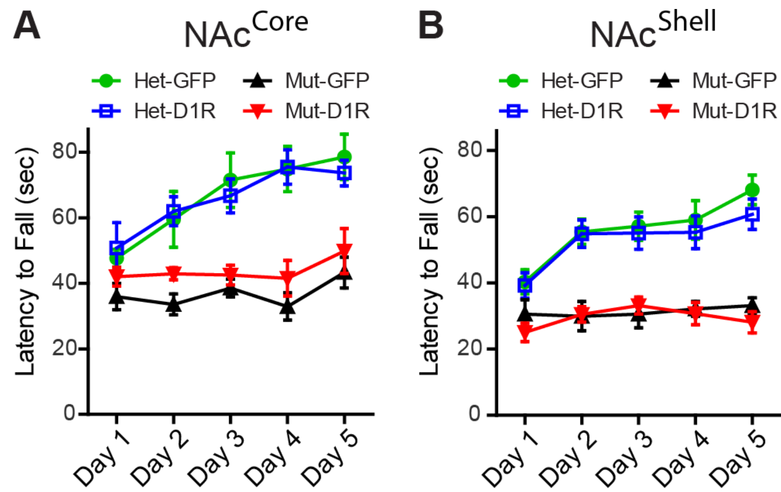

Figure 5.

Neither D1R in the NAc^{Core} nor the NAc^{Shell} improves rotarod performance.

A,B, Average latency to fall during three trials per day of rotarod testing over five days. Neither D1R-NAc^{Core} nor D1R-NAc^{Shell} mice demonstrated significant improvement relative to mutant control groups (NAc^{Core}: Het-GFP, n=8; Het-D1R, n=8; Mut-GFP, n=7; Mut-D1R, n=7; NAc^{Shell}: Het-GFP, n=13; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). Mean ± s.e.m.

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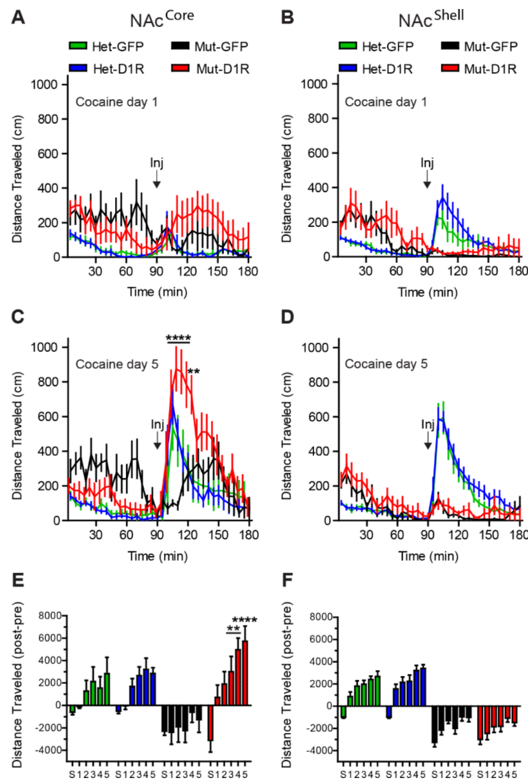

Figure 6.

D1R in the NAc^{Core}, but not the NAc^{Shell}, is sufficient for locomotor sensitization to cocaine.

A,B, Locomotor response to cocaine on Day 1 for NAc^{Core} and NAc^{Shell} mice (NAc^{Core}: Het-GFP, n=7; Het-D1R, n=8; Mut-GFP, n=7; Mut-D1R, n=7; NAc^{Shell}: Het-GFP, n=12; Het-D1R, n=13; Mut-GFP, n=7; Mut-D1R, n=9). ***C,D***, Sensitized locomotor response to cocaine on Day 5 for D1R-NAc^{Core} but not D1R-NAc^{Shell} mice. ***E,F***, Normalized cumulative locomotor activity, [90-minute post-injection period] – [90-minute baseline pre-injection period] for saline (S) and five days of cocaine. ***C,E***, Bonferroni's multiple comparison test, **$p<0.01$, ****$p<0.0001$, D1R-NAc^{Core} or D1R-NAc^{Shell} mice vs D1R mutants. Mean ± s.e.m.