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ORIGINAL INVESTIGATION

Viral restoration of dopamine signaling to the dorsal striatum restores instrumental conditioning to dopamine-deficient mice

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Abstract
Introduction Instrumental responding was evaluated to determine whether mice lacking dopamine [dopamine-deficient mice (DD mice)] could learn to preferentially press a visually cued, active lever for food reward over an inactive lever.
Results When DD mice were treated with 3,4-L-dihydroxyphenalanine (L-dopa) to restore dopamine signaling systemically, they were able to learn to press the active lever as well as control mice, whereas mice lacking dopamine would not perform the task. Importantly, DD mice treated with caffeine (to stimulate locomotor and feeding behaviors) also failed to show preference for the active lever and were slower to retrieve rewards after making a reinforced operant response. Selective restoration of dopamine signaling to the nigrostriatal pathway of DD mice via viral-mediated gene transfer completely restored learning and performance of this simple instrumental task. Furthermore, the virally treated DD mice were willing to lever press as much as control mice for reward in progressive-ratio and high fixed-ratio schedules of reinforcement.
Conclusion These results suggest that the deficit in goal-directed behavior observed in mice without dopamine signaling is the result of decreased motivation to obtain reward, and that dopamine signaling in the dorsal striatum is sufficient to restore normal goal-directed behavior on a variety of operant responding tasks.

Keywords Associative learning · Caffeine · Operant conditioning

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Introduction

It is well established that a hungry, motivated animal will learn to engage in behaviors that allow it to procure food. The organization of complex reward-related behaviors is dependent on a number of components including physiological state, reward expectation, associative learning, and hedonics that act in concert to produce specific behaviors. There are substantial neurobiological data that indicate that the neurotransmitter dopamine is involved in the regulation of reward-related, goal-directed behaviors. For example, during appetitive associative learning tasks, in which animals learn to associate previously neutral stimuli (such as a tone) with reward, dopamine is transiently released in the striatum in response to conditioned and/or unconditioned stimuli. Consistent with this idea, dopamine-depleted animals perform poorly on some aspects of associative learning while performance of other aspects persists (Berridge and Robinson 1998, 2003; Cheng et al. 2003; Datla et al. 2002; Roitman et al. 2004; Salamone and Correa 2002). Electrophysiological recordings made from dopamine neurons in behaving primates also support a role

for dopamine in reward-related associative learning; these findings are formalized in the associative learning hypothesis (Hollerman and Schultz 1998; Ljungberg et al. 1992; Schultz 1998, 2002). Specifically, Schultz and colleagues showed that, in untrained monkeys, random and unexpected presentations of juice rewards (unconditioned stimuli) on the tongue correlated with bursts of electrophysiological activity (action potential spikes) from 60–80% of dopamine neurons recorded. However, after repeated cue–reward pairings, dopamine neuron activation occurred in response to the conditioned cue instead of during presentation (and consumption) of the reward itself. These correlative electrophysiological studies provide support for the idea that midbrain dopamine neurons report about the expectation of reward during associative learning.

Although widely accepted, there are important challenges to the dopamine hypothesis of associative learning. First, a number of studies have revealed that motivational states, and in turn, goal-directed behaviors, are influenced by dopamine signaling (Berridge and Robinson 1998; Wise 2004). For example, when sated animals receive rewards, dopamine release in the nucleus accumbens (NAc) is attenuated compared to that observed in hungry animals (Ahn and Phillips 1999). In addition, hyperdopaminergic tone increases the frequency of goal-directed behaviors during a Pavlovian approach task (Pecina et al. 2003). Another striking example of the influence of dopamine signaling on motivation is that genetically engineered mice that are unable to synthesize dopamine will starve to death even in the presence of highly palatable food that is available ad libitum, but they will readily consume food when dopamine levels are restored (Zhou and Palmiter 1995). These data are consistent with the incentive salience hypothesis of dopamine function postulated by Berridge and Robinson (1998). The hypothesis builds upon previous motivational theories of incentive learning (Bindra 1974; Bolles 1978; Toates 1986) and specifies that dopamine-related signaling is necessary for *attribution of incentive salience* to stimuli, but is not necessary for animals to *learn the association* between conditioned and unconditioned stimuli. In their words, incentive salience "transforms the brain's neural representations of conditioned stimuli, converting an event or stimulus from a neutral 'cold' representation (mere information) into an attractive, and wanted incentive that can grab attention" (p 313). Second, the dopamine hypothesis of associative learning specifies that dopamine neurons provide a teaching signal specific to learning about *appetitive* stimuli, yet some studies report increased dopamine release and/or dopamine neuron firing in response to *aversive* stimuli, suggesting a more complex role for dopamine during associative learning (Horvitz 2000; Horvitz et al. 1997; Salamone 1994; Salamone et al. 2005). A third and, perhaps, most striking challenge to the associative learning hypothesis of dopamine function is that a few studies have revealed that both aversive and appetitive associative learning can occur in the absence of dopamine signaling (Berridge and Robinson 1998; Denenberg et al. 2004; Robinson et al. 2005). For example, dopamine-deficient (DD) mice are able to learn the location of a food reward in a T-maze task designed to measure specific components of goal-directed behaviors including reward seeking, reward consumption, and associative learning (Robinson et al. 2005).

For this study we chose to revisit the issue of whether dopamine signaling is necessary for appetitive associative learning to occur. To this end, we employed an instrumental conditioning task to ascertain whether DD mice could learn to prefer an active, cued lever that resulted in delivery of food reward compared to an inactive, nonreinforced lever. An important constraint when working with dopamine-depleted animals is that they are hypoactive and hypophagic; thus, determining whether they can learn (as opposed to perform) a task can be difficult to extract. To circumvent this issue, our behavioral paradigm was designed to separate performance factors from cognitive processes. Similar approaches were successfully employed to test DD mice in an aversive water-maze task, a conditioned place preference paradigm, and an appetitive T-maze task (Denenberg et al. 2004; Hnasko et al. 2005; Robinson et al. 2005).

To conduct these instrumental conditioning experiments, we used three methods to restore locomotor behavior in DD mice. The first was to restore endogenous dopamine signaling through systemic administration of L-3,4-dihydroxyphenylalanine (L-dopa), which is presumably taken up by dopamine neurons, converted to dopamine, packaged into vesicles, and released in a behaviorally relevant manner throughout the dopaminergic system. L-dopa treatment permits the mice to move and eat until the L-dopa and resulting dopamine are degraded (i.e., for a few hours, depending on the dose administered), after which they return to their dopamine-depleted, hypoactive, hypophagic state (Szczypka et al. 1999; Zhou and Palmiter 1995). The second method was to treat DD mice with caffeine, an adenosine receptor antagonist, which stimulates DD mice to eat and locomote (Kim and Palmiter 2003). The effects of caffeine are mimicked by a selective adenosine A2a receptor antagonist. Because A2a receptors are enriched on medium spiny neurons expressing dopamine D2 receptors, it is possible that the behavioral effects of caffeine are mediated by direct actions in the striatum. Nevertheless, the actions of caffeine are distinct from those of L-dopa (Kim and Palmiter 2003); most importantly, caffeine is unlikely to duplicate the phasic activation of receptors that is achieved by regulated release of dopamine when DD mice are given L-dopa. The third method was to

restore dopamine in DD mice using a viral-mediated, gene-transfer strategy. This technique has the distinct advantage of restoring dopamine signaling to discrete neural circuits (in contrast to the systemic dopamine production achieved by L-dopa administration). Viral-rescued DD flox'd-stop (vrDDfs) mice were included in this study because previously we found that performance deficits in a different associative learning task (the T-maze task) were rescued by a similar viral treatment (Robinson et al. 2006).

Three experiments were conducted to assess the role of dopamine during instrumental learning using a two-lever discrimination task. The first experiment was conducted to determine if restoration of endogenous dopamine signaling in DD mice (via systemic L-dopa treatment) permits these genetically engineered mice to perform the operant task similarly to their littermate controls. The second experiment tested whether dopamine signaling is necessary for acquisition of an associative learning task by testing DD mice in the operant chambers with and without dopamine signaling. The third experiment tested whether restoration of dopamine signaling selectively to the nigrostriatal circuit is sufficient for instrumental conditioning. The results reveal that mice without dopamine (caffeine-treated) are unable to learn this instrumental task, whereas site-specific restoration of dopamine within the nigrostriatal pathway is sufficient to rescue the behavior. Moreover, mice with dopamine signaling restored predominantly to the dorsal striatum are willing to work as hard as control mice when many lever presses are required to earn rewards.

Materials and methods

Apparatus

Standard mouse operant chambers (model ENV-300, Med Associates, Georgia, VT, USA) equipped with fans and housed in sound-attenuating chambers were used to measure instrumental conditioning in mice. Each chamber was equipped with a house light, a sound generator, and two ultrasensitive retractable levers separated by a food receptacle equipped with an infrared head-entry detector. Three 7.9-mm light-emitting diode-cue lights were located above each lever. Auditory stimuli were delivered via a sonalert located on the wall opposite the levers. A computer equipped with the MED-PC IV program (Med Associates) controlled the apparatus and recorded lever presses and head entries into the magazine.

Experiment 1

The DD mice ($Th^{-/-}$, $Dbh^{Th/+}$) used for experiments 1 and 2 were generated by the inactivation of the tyrosine hydroxylase (*Th*) gene in dopamine neurons and restoration of *Th* gene expression in noradrenergic neurons by targeting the *Th* gene to the dopamine beta-hydroxylase (*Dbh*) locus (Zhou and Palmiter 1995). Three- to 4-month-old male DD (n=12) and control (n=12) mice on a mixed C57BL/6×129/SvEv background were used for this study. Controls included littermates with at least one intact *Th* and one intact *Dbh* allele, which is sufficient to maintain normal catecholamine levels (Rios et al. 1999; Thomas et al. 1998). Home cages were kept on heating pads due to food restriction and the reduced L-dopa dose (25 mg/kg) that the mice received during this study. Mice in all three experiments were individually housed and maintained at approximately 85% of ad libitum body weight. Water was available at all times in their home cages. All testing took place during the light phase of the 12-h light/dark cycle. Mice were fed their daily allotment at the end of each behavioral session. Animals in all experiments were treated in compliance with the ethical standards established by the University of Washington Institutional Animal Care and Use Committee.

The purpose of experiment 1 was to establish whether L-dopa-treated DD mice could perform the two-lever discrimination task similarly to controls. At the start of testing, the fan was turned on and individual mice were placed into the operant chambers for a single overnight session. When all operant boxes were loaded with mice, the house light was illuminated and 15 pellets were delivered noncontingently over 15 min. After this magazine training, two levers were extended on either side of the food receptacle. Two cue lights blinked above one lever (designated as the active lever). Depression of the active lever resulted in the delivery of one 20-mg food pellet (Dustless Precision Pellets, BioServe, Frenchtown, NJ, USA) in conjunction with two auditory beeps (two 200-ms, 70-dB, 1,700-2,300-Hz tones separated by a 100-ms interstimulus interval). A 2-s timeout followed each reinforced lever press. An inactive lever press resulted in a 20-s timeout, during which the house and cue lights (above the active lever) were extinguished and lever presses on the active lever did not result in reinforcement delivery. After 9 h, the house light was extinguished and the levers were retracted. Mice were removed from the chambers the following morning. Mice were tested for an additional 4 days (20-min sessions each day) using the same parameters. Chambers were sanitized with 70% ethanol after each test session. The numbers of lever presses, the latencies to obtain pellets, and the percentages of reinforcements consumed were recorded.

Experiment 2

This experiment was designed to test whether mice without dopamine can learn to associate a cued, active lever with reinforcement. However, because we were interested in the

associative learning component, and not the motor component of the task, it was first necessary to pretrain the mice to learn to lever press and obtain food from the reinforcement receptacle. During the pretraining, DD mice (n=10) were treated with L-dopa (25 mg/kg, 30 min prior to test) and exposed to the operant chambers for seven daily training sessions. At the start of each experimental session, the fan was turned on and animals were placed into the testing chamber. When all operant boxes were loaded with mice, the house light was illuminated and two levers were extended on either side of the reinforcement receptacle. Depression of either the right or left lever resulted in the delivery of one 20-mg food pellet. A 2-s timeout, in which neither lever was active, followed each reinforced lever press. Importantly, no auditory or visual stimuli were presented during the pretraining. These seven pretraining sessions for the DD mice lasted either 3 h or until 200 lever presses were made. At the completion of a session, the house light was extinguished and the levers were retracted. A 15-min background magazine program, in which a maximum of 15 pellets were delivered noncontingently on a 30-s variable interval schedule, was run concurrently with the fixed ratio-one (FR1) reinforcement schedule as needed during this pretraining phase (between one and four sessions per subject). Any animals that did not reach criterion (200 lever presses in two consecutive test sessions) were excluded from the study.

Upon completion of the pretraining, DD mice were separated into two similar groups based on the number of lever presses and the length of time required to complete the pretraining task (on day 7) and were subjected to a two-phase, two-lever discrimination task. At the start of each of 24 daily experimental sessions (12 sessions during phase 1 and 12 sessions during phase 2), the fan was turned on and animals were placed into the testing chamber. When all operant boxes were loaded with mice, the house light was illuminated and two levers were extended on either side of the food receptacle. During this phase, two cue lights blinked above one lever (designated as the active lever). Depression of the active lever resulted in the delivery of one 20-mg food pellet (FR1 schedule) in conjunction with two auditory beeps (two 200-ms, 70-dB, 1,700–2,300-Hz tones separated by a 100-ms interstimulus interval). A 2-s timeout followed each reinforced lever press. An inactive lever press resulted in a 20-s timeout, during which the house and cue lights (above the active lever) were extinguished and lever presses on the active lever did not result in reinforcement delivery. At the completion of each 20-min test session, the house light was extinguished and the levers were retracted. Several hours after each daily test session, all DD mice were injected with a low maintenance dose of L-dopa (25 mg/kg) and given access to food and water.

During phase 1, DD mice were tested in two conditions: one group was tested with endogenous dopamine signaling (L-dopa-treated group, n=5, 25 mg/kg given i.p. 30 min prior to testing) and one group was tested without dopamine signaling (caffeine-treated group, n=5, 25 mg/kg given i.p. 30 min before testing). The data generated by these mice during phase 1 testing are represented as the LD1 and CAF1 groups, respectively. If the caffeine group demonstrated a learning curve (developed a preference for the active lever), we could conclude that learning was possible without dopamine. If, however, the caffeine group did not show a preference (and the L-dopa group did), we would not be able to conclude whether the caffeine group learned to discriminate between active and inactive levers but could not perform the task or if learning was actually impaired. Phase 2 was therefore conducted to address whether performance deficits masked learning during phase 1. Thus, during phase 2, all mice were treated with L-dopa (25 mg/kg, administered 30 min prior to test) and tested in the same manner for an additional 12 days. Mice that were treated with L-dopa during phase 1 and L-dopa during phase 2 are referred to as the LD1–LD2 group and mice that were treated with caffeine during phase 1 and L-dopa during phase 2 are referred to as the CAF1–LD2 group. A simple comparison of the learning curves generated during phases 1 and 2 can be made to determine if animals learned to discriminate between active and inactive levers in the absence of dopamine signaling. Specifically, if the caffeine group (CAF1) learned the discrimination in phase 1 but were hindered by performance deficits (and thus could not express a preference), then, during phase 2 (when dopamine is restored), the CAF1–LD2 group should perform better than the L-dopa-treated group did during phase 1 (LD1 group). In other words, during phase 2 testing, when dopamine signaling is restored, the DD mice that were previously treated with caffeine should show robust preference for the active lever on the first and all subsequent test sessions. In contrast, if the CAF1 group did not show a preference during phase 1 simply because they failed to learn to discriminate between the active and inactive levers, then their learning curve during phase 2 should not differ significantly from the learning curve generated by the L-dopa-treated group during phase 1 (i.e., they should show a gradual learning curve across test days 13 through 24 because they are undergoing initial learning of the task as if they had never been exposed to the active and inactive lever contingencies).

Experiment 3

For this experiment, a new line of flox'd-stop DD mice (DDfs) was used. These mice have a nonfunctional *Th* gene due to insertion of a Neo^R gene flanked by lox P sites into

the first intron of the *Th* gene (Hnasko et al. 2006). As with DD mice, these DDfs mice have <1% normal brain dopamine content, severe hypoactivity and aphagia, and they die unless maintained with a daily injection of L-dopa (50 mg/kg). To restore dopamine specifically to the nigrostriatal system, male DDfs (n=13) and control (n=11) mice were injected with a canine adenovirus (CAV-2) engineered to express Cre recombinase (CAVCre) into the dorsal striatum. This procedure restores normal *Th* gene expression to midbrain dopamine neurons that project to this injection site because CAV-2 efficiently transduces axon terminals and is retrogradely transported to neuronal cell bodies (Kremer et al. 2000; Soudais et al. 2001). Bilateral injection of CAVCre into the dorsal striatum restores feeding and normalizes locomotion in these otherwise dopamine-depleted mice (Hnasko et al. 2006).

Mice were trained as described for experiment 2 with the exception that the criteria for inclusion in the study was that the mice were required to make 50 reinforced lever responses in two consecutive 2-h sessions (instead of 200 reinforced responses in two consecutive 3-h sessions). Two-lever discrimination testing was identical to that described in experiment 2.

Experiment 4

After the two-lever discrimination testing, some vrDDfs (n=7) and control (n=7) mice were also tested with a progressive ratio (PR) schedule and a series of FR schedules to examine whether their motivation to work for food rewards was impaired when dopamine signaling was restricted to the striatonigral pathway. Mice were pretrained on a two-lever FR5 schedule of reinforcement for 3 days. The active and inactive lever contingencies were the same as those described for the two-lever discrimination task. After pretraining, five daily sessions of time-constrained, one-lever PR testing were conducted. During the PR testing, only the active lever was extended. The number of lever presses required for reinforcement delivery increased according to a nonarithmetic schedule that increased response requirements in the following way:

Response requirement:	1	2	4	6	9	12	15	19	22	26	30	35	40	45	50	55	61	67…
Incremental increase:		1	2	2	3	3	3	4	4	4	4	5	5	5	5	5	6	6…

Total lever presses and breakpoint (the highest ratio completed in a 90-min session) were measured. After PR testing, the same group of vrDDfs mice was also tested daily on a series of FRs, modeled after an experiment in rats with NAc 6-hydroxy dopamine (6-OHDA) lesions (Salamone et al. 2001). One-lever FR sessions lasted 20 min and ratio requirements/number of reinforcers delivered per completion of a response requirement were administered in the following order: FR5/1, FR20/1, FR50/1, FR100/2, FR200/4, and FR300/6. Mice were tested on each FR contingency for three consecutive days. Total lever presses and the number of reinforcers earned and consumed per session were recorded.

Results

Experiment 1

To evaluate whether L-dopa-treated DD and control mice learned to show preference for the active lever, repeated-measures analysis of variance (RM-ANOVA) was conducted on the number of active lever presses per 20-min session for days 1–4 for both genotypes. This analysis revealed a main effect of day, $F(3, 66)=21.8$, $p<.001$, but no significant effect of genotype nor a day×genotype interaction, suggesting that L-dopa-treated DD and control mice learned the task equivalently across days (Fig. 1a). In addition, L-dopa-treated DD and control mice did not differ in the percentage of rewards consumed (Fig. 1b). These data suggest that L-dopa-treated DD mice can learn and perform a simple two-lever discrimination task similarly to control mice. The mean latency to retrieve reward was <5 s during all sessions and nearly all rewards were consumed (data not shown).

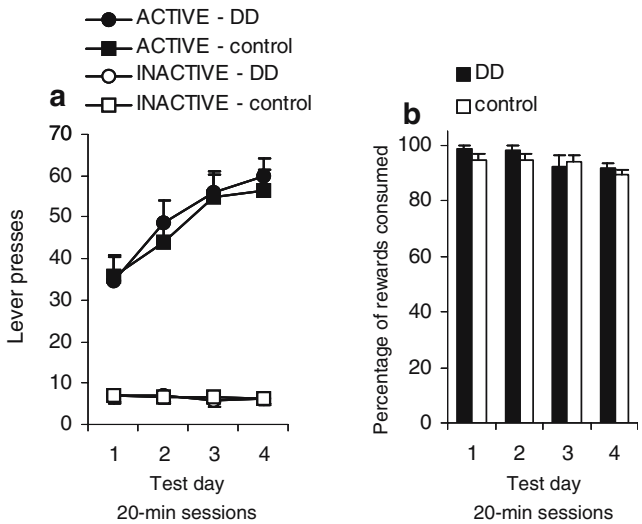

Fig. 1 Comparisons of the operant responses of control and L-dopa-treated DD mice. Control and L-dopa-treated DD mice **a** have similar numbers of lever presses to cued and uncued levers and **b** consume the same number of food pellets on each of four test days (20-min sessions) following a 9-h training session. Results are shown as means±SEM; n=12 mice/group

Experiment 2

After 7 days of pretraining, all L-dopa-treated DD mice reached the criteria of making 200 reinforced lever responses in 3 h or less (data not shown). DD mice were divided into two groups based on the number of lever presses and the latency to complete the pretraining session and treated with either caffeine (CAF1 group) or L-dopa (LD1 group) and tested for 12 days for acquisition of a two-lever discrimination task. Figure 2a shows that during phase 1, DD mice with dopamine (LD1 group) had a learning curve with the number of active lever presses increasing across test days similar to experiment 1.

Some differences in the lever-pressing behavior of L-dopa-treated DD mice were observed between experiments 1 and 2 that may be attributed to intentional differences in experimental design. First, in experiment 1, L-dopa-treated DD mice made more lever presses during the initial days of testing than did L-dopa-treated DD mice during experiment 2. This difference may be explained by the fact that during experiment 1, mice were given a single, overnight exposure to the operant chambers prior to the first day of testing, whereas mice in experiment 2 were not. Second, L-dopa-treated DD mice in experiment 2 showed a steeper learning curve across test days than did L-dopa-treated DD mice in experiment 1. The difference in acquisition rate between experiments 1 and 2 is likely a combination of the overnight exposure and the additional preconditioning phase included in experiment 2 in which both levers were active (i.e., responding on either lever resulted in delivery of reinforcement), which, in effect, required mice to extinguish their operant responding on one lever during subsequent phases of testing in experiment 2. In contrast to the LD1 group, the CAF1 group did not show improvement in the number of active lever presses across days during phase 1 (Fig. 2b). RM-ANOVA was conducted on the number of active vs inactive lever presses per 20-min session across days 1–12 for each drug treatment (caffeine or L-dopa) and revealed a main effect of day, $F(11, 44)=5.1$, $p<.001$, and a main effect of lever, $F(1, 44)=9.9$, $p<.05$, for the LD1 group, but no main effects were observed for the CAF1 group, suggesting that L-dopa-treated DD mice learned to discriminate between the active and inactive levers but the caffeine-treated DD mice did not. There were no statistically significant differences in the number of inactive lever presses per 20-min session for either group during phase 1 (or phase 2). These data suggest that dopamine deficiency impairs acquisition of this instrumental conditioning task. However, because of potential performance deficits, it is premature to conclude from these data alone that caffeine-treated DD mice failed to learn the association between the active lever and reward during phase 1.

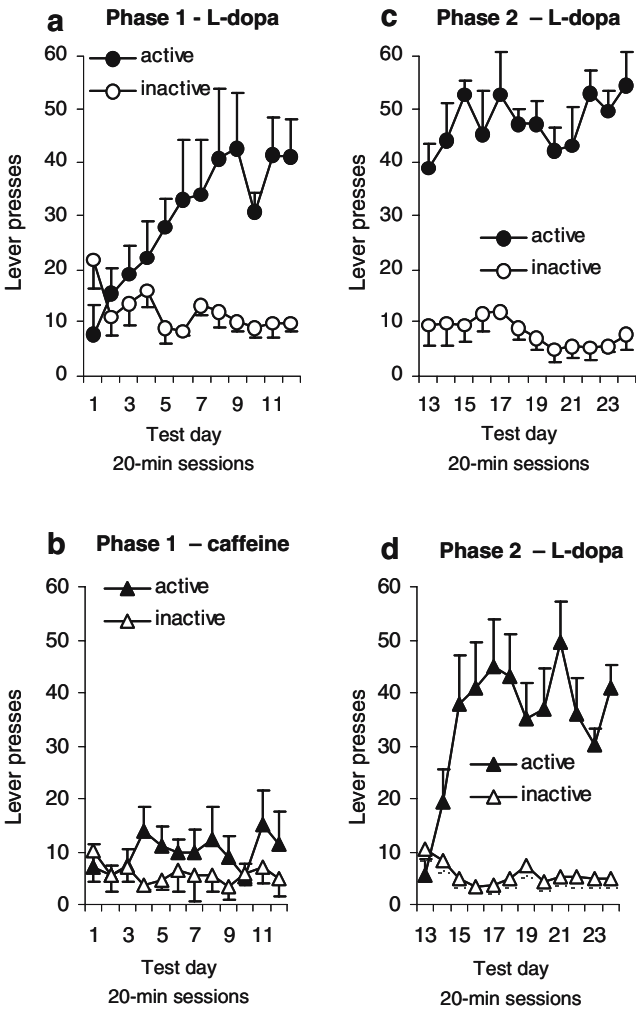

Fig. 2 Comparisons of operant responses of L-dopa-treated and caffeine-treated DD mice. There were 7 days of pretraining, during which pressing either of two levers delivered a food pellet. The experiment had two additional phases: during the first 12 days (**a**, **b**, phase 1) the mice were injected with either L-dopa (LD1 group) or caffeine (CAF1 group), placed in the operant chamber, and then tested for 20 min on a two-lever discrimination task. During this task, two levers were extended; one was active (cued by a blinking light) and the other was inactive. Depression of the active lever resulted in delivery of one 20-mg food pellet on an FR1 schedule of reinforcement. Depression of the inactive lever resulted in a 20-s time-out, during which the house light was extinguished and depression of either lever had no effect. During phase 2 (**c**, LD1–LD2 group; **d**, CAF1–LD2 group), all the mice were given L-dopa and tested as in phase 1. The number presses±SEM on each lever is shown; $n=5$ mice/group

During phase 2 (test days 13 through 24), all DD mice were treated with L-dopa and tested in the operant chambers for 12 more 20-min sessions. The mice that were previously treated with caffeine (CAF1 group) during phase 1 are referred to as the CAF1–LD2 group and the mice that were previously treated with L-dopa (LD1 group) are referred to as the LD1–LD2 group. The active and inactive lever positions and presence and location of the cue lights

were identical between phases. The function of phase 2 was to permit comparison of the performance of L-dopa-treated DD mice during phase 1 (the LD1 group) with the performance of the caffeine–L-dopa-treated mice (the CAF1–LD2 group) during phase 2 to ascertain whether learning (that may have been masked by performance deficits) took place during phase 1. Figure 2c shows that during phase 2, the LD1–LD2 group continued to show strong preference for the active lever during all 12 days of testing, suggesting that this group of mice learned the task fully during phase 1. In contrast, a RM-ANOVA conducted on the number of active lever presses per 20-min session by the LD1 mice during phase 1 compared to the number of active lever presses per 20-min session made by the CAF1–LD2 group during phase 2, revealed a main effect of day, $F(11, 88)=10.7$, $p<.001$, and a day-by-drug treatment interaction $F(11, 88)=2.1$, $p<.05$, but no main effect of drug treatment (i.e., compare the active lever responses from Fig. 2a to those in Fig. 2d). These data suggest that mice that were treated with caffeine during phase 1 and subsequently treated with L-dopa during phase 2 learned to associate the active lever with reward during phase 2, not during phase 1 testing. If the CAF1 group had learned the two-lever discrimination during phase 1, then we would predict to see near asymptotic performance on the first and all subsequent days of phase 2 testing (as with the LD1–LD2 group). The main effect of day combined with the day×treatment interaction indicates that these groups did not change similarly across test days. Inspection of the data reveals that the learning curve generated by the CAF1–LD2 group during phase 2 was steeper than that generated by the LD1 group during phase 1. These data leave open the possibility that during phase 1, the caffeine-treated animals may have made some reward-related associations and thus learned the task more quickly (than the controls) during phase 2 (when dopamine signaling was restored). However, overall, these data suggest that dopamine signaling is necessary for acquisition of this instrumental conditioning task.

The latencies to consume reward after a reinforced response for phases 1 and 2 are shown in Fig. 3a and b, respectively. To evaluate whether L-dopa-treated DD mice and caffeine-treated DD mice were similarly motivated to obtain reward, RM-ANOVA was conducted on the latency to retrieve reward after making a response on the active lever per 20-min session across days 1 though 12 and revealed a main effect of drug treatment, $F(11, 88)=12.7$, $p<.005$, and a main effect of day $F(11, 88)=2.4$, $p<.01$. Inspection of the latency data revealed that CAF1 group (phase 1, Fig. 3a) took progressively longer across days to obtain reward after making a response on the active lever, whereas the LD1 group approached the reinforcement receptacle in <5 s, on average, after making a reinforced

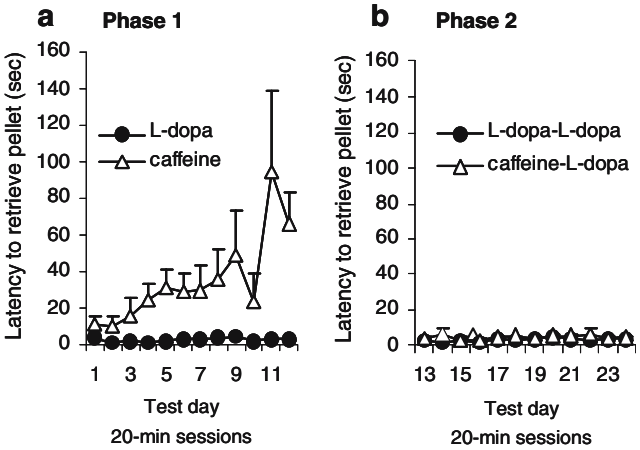

Fig. 3 Latency to retrieve reinforcement after making an operant response on the active lever during phase 1 (**a**) and phase 2 (**b**) of the two-lever discrimination task described in Fig. 2

response on all days. In contrast, during phase 2, all DD mice [those that had been previously treated with caffeine during phase 1 (the CAF1–LD2 group) and those that had been treated with L-dopa during phase 1 (the LD1–LD2 group)] approached the reinforcement receptacle in <5 s on average after making a reinforced response. Both groups consumed 100% of the rewards (data not shown).

Experiment 3

After 7 days of pretraining, all vrDDfs and control mice reached the criteria of making 50 reinforced lever responses in 2 h or less (data not shown). Figure 4 shows that both vrDDfs and control groups had learning curves with the number of active lever presses increasing across test days [RM-ANOVA; main effect of day, $F(9, 198)=20.3$, $p<.001$ (similar to experiment 1)] and the number of inactive lever presses decreasing across days [RM-ANOVA; main effect of day, $F(9, 198)=48.9$, $p<.001$]. The mean latency to consume rewards was <5 s for both groups of mice and all rewards were retrieved and consumed (data not shown). These data suggest that restoration of dopamine selectively to the nigrostriatal pathway is sufficient to restore acquisition of this instrumental task in DD mice.

Experiment 4

Subsets of vrDDfs and control mice were subsequently tested in a PR experiment to assess their motivation to work for rewards. RM-ANOVA conducted on the number of lever presses made by vrDDfs and control mice per 90-min session across days 1 through 5 revealed a main effect of day, $F(4, 48)=2.9$, $p<.05$, but no effect of viral treatment. Thus, vrDDfs and control mice were similar on the highest ratio completed (breakpoint) across all 5 days of one-lever PR testing (Fig. 5a). Furthermore, as shown in Fig. 5b,

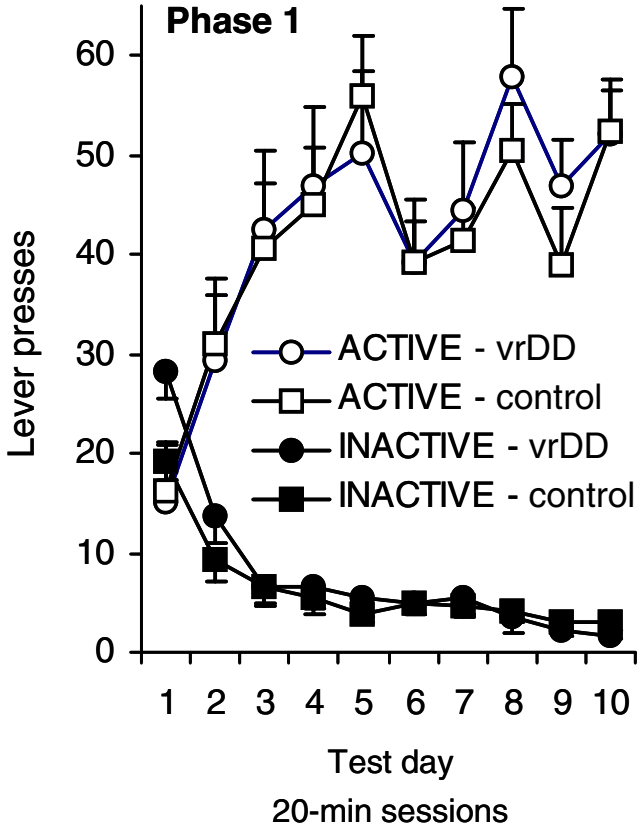

Fig. 4 Comparisons of operant responses of control mice (n=11) and vrDDfs mice (with dopamine signaling selectively restored to the dorsal striatum, n=13) during performance of a two-lever discrimination task. There were 7 days of pretraining, during which pressing either of two levers resulted in the delivery of reward on an FR1 schedule of reinforcement. Then, the mice were tested for 10 days (20 min per session) on a two-lever discrimination task, in which depression of the active lever (cued by a blinking light) resulted in the delivery of a food pellet to the reinforcement receptacle. The mean number±SEM of lever presses on each lever by the two groups of mice is shown

vrDDfs and control mice adjusted their work output similarly across a succession of 20-min FR sessions [RM-ANOVA; main effect of day, $F(17, 204)=20.3$, $p<.001$), but no main effect of viral treatment].

Discussion

The purpose of these experiments was to determine the role of dopamine during instrumental conditioning. Experiment 1 established that L-dopa-treated DD mice performed similarly to controls on a two-lever discrimination task for food reinforcement. Thus, there are no developmental defects as a consequence of dopamine deficiency that would preclude mastery of this simple task when dopamine signaling is restored. In contrast, experiment 2 established that although caffeine-treated DD mice were able to execute the motor patterns to make the operant responses, these mice were *unable* to learn to show preference for an active, cued lever. Experiment 3 revealed that the restoration of dopamine signaling selectively in the nigrostriatal pathway was sufficient to rescue goal-directed behavior on this relatively simple instrumental conditioning task. Experiment 4 showed that vrDDfs mice are willing to work as hard as control mice are to obtain rewards.

We observed that mice without dopamine were unable to show preference for the active, cued lever during this instrumental conditioning task. DD mice treated with caffeine failed to complete a relatively simple operant task that required them to depress a cued, active lever to obtain food reward. Importantly, these mice also took longer to obtain reinforcement after the operant response was made. Because DD mice treated with caffeine have the motor ability to eat, lever press, and move about the operant chamber, gross sensory and motor deficits cannot explain the failure of DD mice to approach the reinforcement receptacle with short latency following an operant response. In addition, caffeine treatment is not a likely explanation for the impaired latency to approach reward because the dose of caffeine (25 mg/kg) that was used produces levels of activity comparable to that of L-dopa-treated DD controls (Kim and Palmiter 2003; Szczypka et al. 1999). Further, DD mice treated with caffeine consumed all reinforcements available, suggesting that the observed deficits are not attributable to decreased food appetite. Nevertheless, it is possible that the dose of caffeine used in these experiments interfered with important aspects of instrumental learning (Angelucci et al. 1999), perhaps by enhancing exploration (Durcan and Lister 1989) or anxiety (Silva and Frussa-Filho 2000).

We considered the possibility that the impairment of DD mice might be a motor-related issue because the caffeine-treated DD mice made approximately half as many lever presses as L-dopa-treated DD mice. However, statistical analysis and examination of the learning curves generated during experiment 2 revealed that the number of operant responses on the inactive lever per 20-min session remained constant regardless of whether the mice were dopamine depleted or repleted. In contrast, the number of operant responses on the active lever was markedly reduced in the absence of dopamine signaling. Thus, our data suggest that goal-directed behavior, but not the ability to execute the operant response, was compromised and are consistent with the suggestion that decreased operant responding for reinforcement during instrumental conditioning may reflect a decrease in motivation, or low goal-expectancy, which results in insufficient behavior to obtain reward (Balleine and Dickinson 1998; Yin et al. 2004).

Previously, we used a T-maze task to distinguish which components of goal-directed behavior are influenced by dopamine signaling. We found that dopamine *is not*

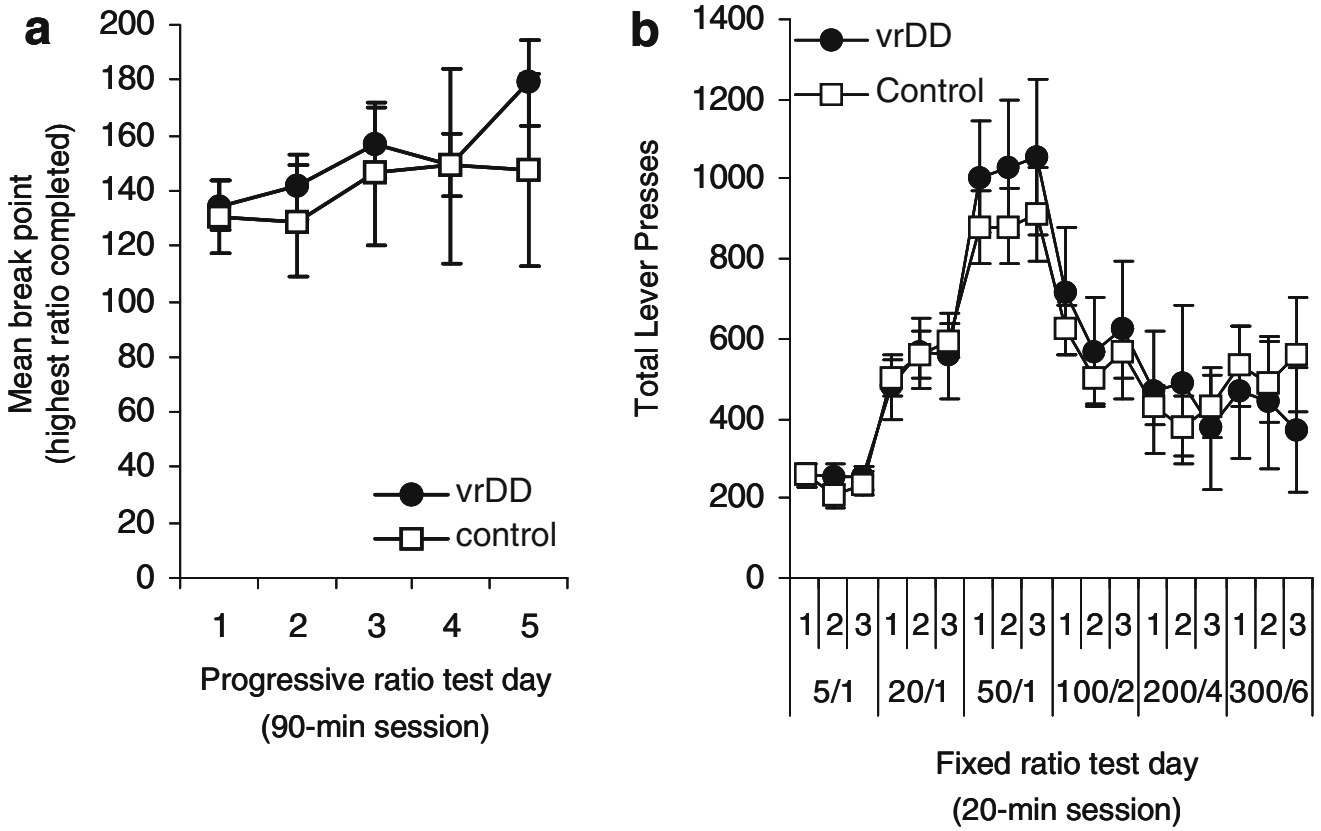

Fig. 5 Comparisons of the operant responses of control and vrDDfs mice on a PR lever-press task (**a**) or various fixed-ratio schedules of reinforcement (**b**). This experiment was performed with a subset of the mice described in Fig. 4. **a** After 3 days of pretraining on a two-lever FR5 schedule of reinforcement, the mice were tested for 5 days (90 min per session) with a PR schedule of reinforcement (see "Materials and methods") and the breakpoint (highest ratio of responding on the active lever completed). **b** Then, the same groups were tested on a series of FRs (number of presses required/number of 20-mg pellets delivered is indicated) for 3 days (20-min sessions). The mean number±SEM of presses on the active lever is shown; $n=7$ mice/group

necessary for mice to learn the location of reward (i.e., learn to associate a place with reward) or for mice to consume reward, but *is necessary* for mice to seek rewards as well as controls. We showed that DD mice on caffeine failed to develop a preference for the arm where rewards were available; instead, they chose which arm to enter at random. However, when they did randomly choose correctly (i.e., when the baited, CS+ arm was entered), they consumed all available rewards. In a second phase of testing, when the caffeine-treated mice were subsequently given L-dopa, they demonstrated that they *had learned* (while on caffeine) which arm had the reward because they chose it preferentially during the first day of phase 2 L-dopa testing (Robinson et al. 2005). Thus, the T-maze results revealed that, in some goal-directed tasks, mice without dopamine signaling can, indeed, learn to associate salient environmental cues with reward. Likewise, in another study, we showed that untreated DD mice can develop a conditioned place preference for morphine, which involves learning to associate a particular compartment of a training chamber with morphine reward (Hnasko et al. 2005). The ability of DD mice to demonstrate associative learning in those tasks but not in this instrumental task is noteworthy and underscores the point that there are multiple subtypes of reward-related learning, which are likely to depend on dopamine signaling to variable degrees.

Unlike our findings with the T-maze task (Robinson et al. 2005), these data are consistent, in part, with both the associative learning hypothesis and the incentive salience hypothesis. The finding that DD mice (treated with caffeine) are unable to show preference for the active, cued lever may be the result of an impaired ability to form reward-related associations in the absence of dopamine signaling and is consistent with theories which describe the role of dopamine in associative learning (Beninger and Miller 1998; Di Chiara 1998). We observed that caffeine-treated DD mice made a lever press and then roamed around the chamber a few times before eventually approaching the reinforcement receptacle. In contrast, L-dopa-treated DD mice consistently made a lever press and then proceeded immediately to the reinforcement receptacle. Perhaps these mice were unable to learn the task because the latency between making the reinforced operant response (active

lever press) and retrieving the food reward was too long for the formation of reward-related associations.

Another possibility is that the failure of DD mice to perform the operant task is primarily due to a lack of motivation to obtain reward, as reflected in the longer latency to obtain reward following an operant response on the active lever compared to controls. In the T-maze task, approaching (but not consuming) the reward after making a correct arm entry was also approximately threefold slower for caffeine-treated DD mice compared to L-dopa-treated DD mice. Thus, in the absence of strong motivation to consume food pellets, it appeared that the mice roamed around the chamber after pressing the lever and found the pellets accidentally. One interpretation of these data that is consistent with the incentive salience hypothesis posited by Berridge and Robinson (1998) is that without dopamine, features of the cued, active lever failed to gain motivational significance, resulting in few operant responses on the active lever and long latencies to retrieve reward. This interpretation is in agreement with a recent report describing the *increased* goal-directed behavior demonstrated by hyperdopaminergic mice, which have elevated synaptic dopamine (Pecina et al. 2003). Specifically, in a straight-alley runway task for sweet reward, hyperdopaminergic mice left the start box more quickly, spent less time pausing in the runway, made fewer investigatory reversals, and less often retraced their steps en route to the reward. Of note, these animals did not show an increased liking of rewards as measured by orofacial responses to intraoral sucrose delivery. Thus, excess dopamine signaling appears to increase the motivation to obtain reward, whereas the absence of dopamine signaling decreases motivation to obtain reward. Restoration of dopamine signaling to the dorsal striatum completely restored the goal-directed behavior in both the T-maze task (Robinson et al. 2006) and in this operant task.

The vrDDfs mice with dopamine signaling selectively restored in the dorsal striatum manifested similar motivation to controls to obtain food rewards on both progressive-ratio and high fixed-ratio schedules of reinforcement. These results suggest that dopamine signaling outside the dorsal striatum is not necessary for incentive to lever-press hundreds of times to obtain a few 20-mg food pellets. These results are inconsistent with findings in which 6-OHDA lesions of the NAc attenuated the performance of rats when they had to lever press many times for rewards (Aberman et al. 1998; Hamill et al. 1999; Robbins et al. 1983; Salamone et al. 2001), as well as data obtained by the infusion of dopamine receptor antagonists into the NAc (Koch et al. 2000; Nowend et al. 2001; Sutton and Beninger 1999). While formally possible, it is unlikely that this discrepancy reflects a difference in the role of dopamine neurons that project to the NAc in mice and rats. Another possibility is that the vrDDfs mice have intact dopamine neurons (compared to 6-OHDA-lesioned rats), and hence, they could continue to release other neurotransmitters including neuropeptides (cholecystokinin and neurotensin) and glutamate (Chuhma et al. 2004; Seutin 2005). These signaling molecules would be lost along with dopamine after 6-OHDA lesions. Behavioral consequences of 6-OHDA lesions wane over time ranging from days to weeks (Ikemoto and Panksepp 1999; Salamone et al. 2001). Because the vrDDfs mice used in this study were tested several months after viral rescue, they may have compensated for loss of dopamine signaling in the ventral striatum during that time, for instance, by responding to other transmitters made by dopamine neurons. Another possibility is that, over a period of months, dopamine signaling in the dorsal striatum can usurp some of the functions normally attributed to dopamine signaling in the ventral striatum. A final consideration is that the viral rescue strategy used for these studies involved injecting CAVCre virus into the dorsal striatum to enable recombination of the *Th* locus in dopamine neurons that project there. We have observed that some ventral tegmental area neurons express tyrosine hydroxylase after this viral treatment, presumably because they send collaterals to the dorsal striatum (Hnasko et al. 2006). While we can detect a small amount of dopamine in the ventral striatum by chemical detection or fast-scan cyclic voltammetry, levels are on par with dopamine concentrations following 6-OHDA lesions. Immunostaining to reveal dopamine receptor sensitivity and TH also suggest very little dopamine signaling is present in the ventral striatum. Nonetheless, although greatly reduced, there may be sufficient dopamine signaling in the NAc of the vrDDfs mice for them to respond normally in progressive-ratio and high fixed-ratio reinforcement paradigms.

The DD mice that are rescued by viral transduction of the dorsal striatum are remarkably normal not only in terms of their motivation to work for food but also in their locomotor activity. Their locomotor activity is enhanced, especially at night, relative to wild-type mice, but they still manifest dramatic L-dopa-induced locomotion (Hnasko et al. 2006). These results are surprising because aspects of locomotor activity, especially psychostimulant-induced activity, are thought to depend on dopamine signaling in the NAc. Nevertheless, the robust activity induced by L-dopa argues that dopamine signaling is greatly attenuated in brain regions of vrDDfs mice where dopamine can stimulate locomotion. Our results demonstrate what vrDDfs mice can do, but the precise mechanisms by which their behaviors are restored are not established. Thus, while the results presented here contrast with a large amount of literature indicating that dopamine signaling in NAc is important for aspects of motivated behavior, further

analysis is necessary to understand the mechanisms responsible for the normal behavior of vrDDfs mice.

In conclusion, we have shown that mice without dopamine are unable to learn to show preference for the active, cued lever in a simple two-lever discrimination task. We surmise that this impairment in goal-directed behavior is the result of decreased motivation to obtain food reward, stemming from a deficit in incentive learning and/or a deficit in attribution of incentive salience during reward learning. Mice were able to complete the instrumental task following selective restoration of dopamine signaling within the nigrostriatal pathway. These results complement and extend our previous findings showing that other goal-directed behaviors, such as reproductive behavior, nest building, reward consumption, and food seeking, are also rescued when dopamine signaling is site-specifically restored in the dorsal striatum (Robinson et al. 2006; Szczypka et al. 2001). Our results suggest that it is necessary to devise more challenging tests to discern the roles of dopamine in the ventral striatum.

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References

Aberman JE, Ward SJ, Salamone JD (1998) Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. Pharmacol Biochem Behav 61:341–348

Ahn S, Phillips AG (1999) Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. J Neurosci 19:RC29

Angelucci ME, Vital MA, Cesario C, Zadusky CR, Rosalen PL, Da Cunha C (1999) The effect of caffeine in animal models of learning and memory. Eur J Pharmacol 373:135–140

Balleine BW, Dickinson A (1998) Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37:407–419

Beninger RJ, Miller R (1998) Dopamine D1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev 22:335–345

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309–369

Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26:507–513

Bindra D (1974) A motivational view of learning, performance, and behavior modification. Psychol Rev 81:199–213

Bolles RC (1978) Reinforcement, expectancy and learning. Psychol Rev 79:394–409

Cheng JJ, de Bruin JP, Feenstra MG (2003) Dopamine efflux in nucleus accumbens shell and core in response to appetitive classical conditioning. Eur J Neurosci 18:1306–1314

Chuhma N, Zhang H, Masson J, Zhuang X, Sulzer D, Hen R, Rayport S (2004) Dopamine neurons mediate a fast excitatory signal via their glutamatergic synapses. J Neurosci 24:972–981

Datla KP, Ahier RG, Young AM, Gray JA, Joseph MH (2002) Conditioned appetitive stimulus increases extracellular dopamine in the nucleus accumbens of the rat. Eur J Neurosci 16:1987–1993

Denenberg VH, Kim DS, Palmiter RD (2004) The role of dopamine in learning, memory, and performance of a water escape task. Behav Brain Res 148:73–78

Di Chiara G (1998) A motivational learning hypothesis of the role of mesolimbic dopamine in compulsive drug use. J Psychopharmacol 12:54–67

Durcan MJ, Lister RG (1989) Does directed exploration influence locomotor activity in a holeboard test? Behav Neural Biol 51:121–125

Hamill S, Trevitt JT, Nowend KL, Carlson BB, Salamone JD (1999) Nucleus accumbens dopamine depletions and time-constrained progressive ratio performance: effects of different ratio requirements. Pharmacol Biochem Behav 64:21–27

Hnasko TS, Sotak BN, Palmiter RD (2005) Morphine reward in dopamine-deficient mice. Nature 438:854–857

Hnasko TS, Perez FA, Scouras AD, Stoll EA, Gale SD, Luquet S, Phillips PE, Kremer EJ, Palmiter RD (2006) Cre recombinase-mediated restoration of nigrostriatal dopamine in dopamine-deficient mice reverses hypophagia and bradykinesia. Proc Natl Acad Sci USA 103:8858–8863

Hollerman JR, Schultz W (1998) Dopamine neurons report an error in the temporal prediction of reward during learning. Nat Neurosci 1:304–309

Horvitz JC (2000) Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. Neuroscience 96:651–656

Horvitz JC, Stewart T, Jacobs BL (1997) Burst activity of ventral tegmental dopamine neurons is elicited by sensory stimuli in the awake cat. Brain Res 759:251–258

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev 31:6–41

Kim DS, Palmiter RD (2003) Adenosine receptor blockade reverses hypophagia and enhances locomotor activity of dopamine-deficient mice. Proc Natl Acad Sci USA 100:1346–1351

Koch M, Schmid A, Schnitzler HU (2000) Role of nucleus accumbens dopamine D1 and D2 receptors in instrumental and Pavlovian paradigms of conditioned reward. Psychopharmacology (Berl) 152:67–73

Kremer EJ, Boutin S, Chillon M, Danos O (2000) Canine adenovirus vectors: an alternative for adenovirus-mediated gene transfer. J Virol 74:505–512

Ljungberg T, Apicella P, Schultz W (1992) Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol 67:145–163

Nowend KL, Arizzi M, Carlson BB, Salamone JD (2001) D1 or D2 antagonism in nucleus accumbens core or dorsomedial shell suppresses lever pressing for food but leads to compensatory increases in chow consumption. Pharmacol Biochem Behav 69:373–382

Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. J Neurosci 23:9395–9402

Rios M, Habecker B, Sasaoka T, Eisenhofer G, Tian H, Landis S, Chikaraishi D, Roffler-Tarlov S (1999) Catecholamine synthesis is mediated by tyrosinase in the absence of tyrosine hydroxylase. J Neurosci 19:3519–3526

Robbins TW, Roberts DC, Koob GF (1983) Effects of d-amphetamine and apomorphine upon operant behavior and schedule-induced

licking in rats with 6-hydroxydopamine-induced lesions of the nucleus accumbens. J Pharmacol Exp Ther 224:662–673
Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119:5–15
Robinson S, Sotak BN, During MJ, Palmiter RD (2006) Local dopamine production in the dorsal striatum restores goal-directed behavior in dopamine-deficient mice. Behav Neurosci 120:196–200
Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM (2004) Dopamine operates as a subsecond modulator of food seeking. J Neurosci 24:1265–1271
Salamone JD (1994) The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behav Brain Res 61:117–133
Salamone JD, Correa M (2002) Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behav Brain Res 137:3–25
Salamone JD, Wisniecki A, Carlson BB, Correa M (2001) Nucleus accumbens dopamine depletions make animals highly sensitive to high fixed ratio requirements but do not impair primary food reinforcement. Neuroscience 105:863–870
Salamone JD, Correa M, Mingote SM, Weber SM (2005) Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. Curr Opin Pharmacol 5:34–41
Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1–27
Schultz W (2002) Getting formal with dopamine and reward. Neuron 36:241–263
Seutin V (2005) Dopaminergic neurones: much more than dopamine? Br J Pharmacol 146:167–169
Silva RH, Frussa-Filho R (2000) The plus-maze discriminative avoidance task: a new model to study memory–anxiety interactions. Effects of chlordiazepoxide and caffeine. J Neurosci Methods 102:117–125
Soudais C, Laplace-Builhe C, Kissa K, Kremer EJ (2001) Preferential transduction of neurons by canine adenovirus vectors and their efficient retrograde transport in vivo. FASEB J 15:2283–2285
Sutton MA, Beninger RJ (1999) Psychopharmacology of conditioned reward: evidence for a rewarding signal at D1-like dopamine receptors. Psychopharmacology (Berl) 144:95–110
Szczypka MS, Rainey MA, Kim DS, Alaynick WA, Marck BT, Matsumoto AM, Palmiter RD (1999) Feeding behavior in dopamine-deficient mice. Proc Natl Acad Sci USA 96:12138–12143
Szczypka MS, Kwok K, Brot MD, Marck BT, Matsumoto AM, Donahue BA, Palmiter RD (2001) Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron 30:819–828
Thomas SA, Marck BT, Palmiter RD, Matsumoto AM (1998) Restoration of norepinephrine and reversal of phenotypes in mice lacking dopamine beta-hydroxylase. J Neurochem 70:2468–2476
Toates FM (1986) Motivational systems. Cambridge University Press, Cambridge
Wise RA (2004) Dopamine, learning and motivation. Nat Rev Neurosci 5:483–494
Yin HH, Knowlton BJ, Balleine BW (2004) Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 19:181–189
Zhou QY, Palmiter RD (1995) Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 83:1197–1209