Molecular Psychiatry (2013), 1–9

www.nature.com/mp

ORIGINAL ARTICLE

Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation

P Trifilieff[1,2], B Feng[3], E Urizar[3], V Winiger[1], RD Ward[1,3], KM Taylor[3], D Martinez[2,3], H Moore[2,3], PD Balsam[2,3,4], EH Simpson[2,3] and JA Javitch[2,3,5]

A decrease in dopamine D2 receptor (D2R) binding in the striatum is one of the most common findings in disorders that involve a dysregulation of motivation, including obesity, addiction and attention deficit hyperactivity disorder. As disruption of D2R signaling in the ventral striatum—including the nucleus accumbens (NAc)—impairs motivation, we sought to determine whether potentiating postsynaptic D2R-dependent signaling in the NAc would improve motivation. In this study, we used a viral vector strategy to overexpress postsynaptic D2Rs in either the NAc or the dorsal striatum. We investigated the effects of D2R overexpression on instrumental learning, willingness to work, use of reward value representations and modulation of motivation by reward associated cues. Overexpression of postsynaptic D2R in the NAc selectively increased motivation without altering consummatory behavior, the representation of the value of the reinforcer, or the capacity to use reward associated cues in flexible ways. In contrast, D2R overexpression in the dorsal striatum did not alter performance on any of the tasks. Thus, consistent with numerous studies showing that reduced D2R signaling impairs motivated behavior, our data show that postsynaptic D2R overexpression in the NAc specifically increases an animal's willingness to expend effort to obtain a goal. Taken together, these results provide insight into the potential impact of future therapeutic strategies that enhance D2R signaling in the NAc.

Molecular Psychiatry advance online publication, 28 May 2013; doi:10.1038/mp.2013.57

Keywords: dopamine; D2 receptor; motivation; nucleus accumbens; reward; viral vector

INTRODUCTION

The mesoaccumbens dopamine (DA) pathway modulates motivation.[1–3] In the nucleus accumbens (NAc), DA mediates its effects through D1- and D2-like receptors. A decrease in D2 receptor (D2R) availability in the striatum, including the NAc, is a common imaging phenotype in disorders that involve the dysregulation of motivation, including obesity,[4] addiction[5] and attention deficit hyperactivity disorder.[6] Moreover, imaging studies in human subjects have shown that striatal D2R levels correlate with characteristics such as sensation seeking and motivation.[7–9] These studies clearly establish the relevance of D2R function to human disorders of motivation, and highlight the importance of determining causal relationships between D2R expression and motivation. One powerful approach for revealing such relationships is to combine in rodents region-specific manipulations of D2R expression with validated methods for measuring specific aspects of motivation.

Changes in motivated behavior can be driven by a number of underlying mechanisms, including how much effort the animal is willing to expend for the reward—that is, primary motivational stimuli or goals[10]—as well as the organism's valuation of a reward.[11–13] Both of these aspects of motivation have been shown to depend on dopaminergic transmission in the NAc. In rodents, willingness to work for a reward is modified by altering D2R signaling in the NAc. Blocking D2Rs in the NAc shifts the animals' choice away from more effortful toward less effortful behavior[11] and the genetic deletion of the D2R also impairs motivated, reward-seeking behavior.[14] Similarly, selective lesioning of the NAc in rats impairs their willingness to work for a reward.[15,16] On the other hand, DA release in the NAc is associated with the animal's valuation of an appetitive stimulus even in the absence of a work requirement to obtain the stimulus.[13]

The aim of this study was to investigate whether increasing postsynaptic NAc D2R levels can enhance motivation, and if so, whether distinct aspects of motivated behavior might be selectively modulated. The D2R was overexpressed in the striatum using viral gene transfer in adult mice. To explore the effect of D2R overexpression on motivation, overexpression in the NAc was compared with that in the caudate/putamen in different groups of mice. Behavior was studied using operant tasks that assess instrumental learning, willingness to expend effort to obtain a goal, the ability to use representations of reward to guide responding and the capacity of reward-associated cues to modulate motivation.

MATERIALS AND METHODS

Viruses

As described previously,[17] adeno-associated viruses 1/2 expressing either (1) $D2_LR$ fused to mVenus or (2) green fluorescent protein (GFP) were used (see Supplementary Information).

Aequorin assay

A functional assay based on luminescence of mitochondrial aequorin following intracellular Ca^{2+} release was performed as described previously.[18]

[1]Department of Neuroscience, Columbia University, New York, NY, USA; [2]New York State Psychiatric Institute, New York, NY, USA; [3]Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY, USA; [4]Department of Psychology, Barnard College, New York, NY, USA and [5]Department of Pharmacology, Columbia University College of Physicians and Surgeons, New York, NY, USA. Correspondence: Dr JA Javitch, 1051 Riverside Drive, Unit 19, New York, NY 10032, USA.
E-mail: jaj2@columbia.edu
Received 12 October 2012; revised 21 March 2013; accepted 2 April 2013

Animals

All procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Columbia University and the New York State Psychiatric Institute. Wild-type female congenic C57Bl/6j mice were used for behavioral experiments (postnatal ages 90 days). D2R knockout (KO)[19] and their C57Bl/6j WT littermates were used for immunohistochemical experiments (see Supplementary Information).

Surgeries/viral injections

Viral injections were performed as described previously.[17] CPu was targeted with two bilateral injections (4 sites total, 0.75 μl virus injected into each): A–P, 1.5 mm and 0; M–L, ± 1.5 mm and ± 2.5 mm; all 3 mm ventral to brain surface. NAc was targeted with a single injection site bilaterally (2 sites total, 0.5 μl virus injected in each site): A–P, 1.7 mm; M–L, ± 1.7 mm; and both 3.8 mm ventral to brain surface. All coordinates given are relative to Bregma. Behavioral testing began 1 month after surgery.

[^{3}H]N-methylspiperone binding assay

Binding assay was performed to investigate the degree of D2R overexpression (See Supplementary Information).

Behavioral testing

Apparatus. The apparatus used has been described in detail in Simpson *et al.*[20] (see Supplementary Information).

Procedures. Seven animals in each group were used in this study, except for the Pavlovian to instrumental transfer (PIT) experiment where there were only six mice in the CPu-GFP group owing to the death of one animal. The same animals performed each of the behavioral procedures.

To assess instrumental learning and willingness to work in face of increasing effort, dipper training, lever pressing training, fixed-interval (FI) training and progressive ratio (PR) testing were performed as described in Simpson *et al.*[20] with some modifications (see Supplementary Information).

Random ratio testing. Random ratio (RR) consists of a constant probability of reinforcement for each lever press. The mice were first trained for 5 days in 1-h RR5 sessions. Animals were then tested in concurrent RR/choice procedure that has been extensively shown to assess effort in instrumental responding.[11] This task consisted of having 8–12 g of lab chow available in a dish in the operant chamber while the mouse worked on the RR schedule. Increasing ratios were used (RR5, RR10 and RR20). A previous study using a preference test showed that evaporated milk serves as a reinforcer for mice using a preference task.[21] For each RR, both concurrent choice and simple RR sessions were repeated in a pseudorandom manner.

Devaluation procedure. This task was used to assess alteration of the outcome value. Mice had free access to either chow or the evaporated milk reward for 1 h in the home cage (single-housed) before a lever press test conducted in a 15-min extinction session. Following this session, the animals received 2 days of retraining using an RR20 procedure before the second test. This test was identical to the first except that the mice were pre-fed with the other outcome. The order of pre-feeding was counterbalanced across subjects within each group.

PIT test. PIT was performed 2 weeks after the final devaluation test. In the Pavlovian training, an auditory stimulus (85 dB, 2000 Hz) served as conditioned stimulus (CS). Pavlovian training consisted in 5 presentations of 2 min CS with a variable intertrial interval (ITI) (mean 8 min) for 5 days. The evaporated milk reward was made available for 30 s with a variable delay after the beginning of the tone-CS (mean 1 min). We limited the Pavlovian training to five sessions as previous studies have shown that overtraining impairs PIT.[22] The PIT test was conducted on the sixth day and consisted of a 10 min extinction, followed by five cycles of 2-min CS presentation with 2 min ITI, without reward delivery.

Histology/immunohistochemistry

Brain tissue preparation, immunohistochemistry, confocal microscopy and image acquisition were performed as described previously.[17] The following primary antibodies were used in this study: home-made rabbit polyclonal anti-D2[17] (1/500) and rabbit polyclonal anti-MAP2 (Abcam, Cambridge, MA, USA; ab32454; 1/2000).

Statistical analysis

Data were analyzed using mixed analyses of variance (ANOVAs) with appropriate terms, followed by *post hoc* Bonferroni comparisons (when appropriate) or *t*-tests. For all the tasks analyzed, the number of lever presses was used as the dependent measure (see also Supplementary Information).

RESULTS

Characterization of D2R-mVenus overexpression

To facilitate visualization of the exogenous D2Rs and discriminate between exogenous and endogenous receptors, we generated a fusion construct of D2R tagged with mVenus at its C-terminus. To verify that the fusion construct was functional, we expressed D2R-mVenus in HEK cells and assessed G-protein activation by the agonist quinpirole using an aequorin-based functional assay (see Supplementary Information). As shown in Figure 1a, fusion of mVenus to the D2R receptor did not alter the ability of the receptor to activate G protein.

We overexpressed D2R-mVenus or GFP alone as a control in either the NAc or the dorsal striatum (CPu) of adult mice using AAV-mediated gene transfer (Figure 1b) and quantified the increase in D2R expression by performing a [^{3}H]N-methylspiperone binding assay. Expression of D2R-mVenus resulted in an approximately 10-fold increase in D2R binding in both NAc (D2R-mVenus: $B_{max} = 17.8 \pm 1.2$ pmol/mg protein, $K_d = 36.3 \pm 10.4$ pM; GFP: $B_{max} = 1.65 \pm 0.15$ pmol/mg protein, $K_d = 28.9 \pm 4.8$ pM) and CPu (D2R-mVenus: $B_{max} = 17.3 \pm 0.5$ pmol/mg protein, $K_d = 36.6 \pm 10.1$ pM; GFP: $B_{max} = 1.64 \pm 0.03$ pmol/mg protein, $K_d = 42.5 \pm 9.8$ pM).

Immunofluorescence experiments revealed that D2R-mVenus had very low somatic expression (Figure 1c) and partially colocalized with the dendritic marker MAP2 (Figure 1d), consistent with the known predominant postsynaptic localization of the receptor.[23] Taken together, these data suggest that our viral overexpression system leads to a large increase in functional D2R with the expected subcellular localization.

The minimal and maximal extents of D2R-mVenus and GFP expression are depicted in the left and right hemispheres, respectively, of coronal sections in Figure 1e. D2R-Venus expression in the NAc largely targeted the core in all animals and partially the shell, with very low spread to the dorsal striatum. D2R-Venus expression in the CPu did not reach the NAc except in one animal (see Figure 1e) and spread throughout roughly 40–70% of the entire CPu. We did not observe significant expression in extrastriatal areas in any of the animals.

D2R overexpression does not alter simple instrumental conditioning

D2R overexpression in the NAc or CPu did not alter learning of instrumental conditioning in the continuous reinforcement schedule compared with GFP overexpression as shown by the number of lever presses (NAc: $F_{(1,\ 12)} = 0.30$, $P = 0.59$; CPu: $F_{(1,\ 12)} = 0.02$, $P = 0.89$) (Figures 2a and b). Previous studies show that fixed interval (FI) training before PR testing creates a sensitive assay of motivation.[24] We analyzed the number of lever presses during FI schedules with increasing intervals (Figures 2a and b). D2R overexpression had no effect at any interval tested (all $Ps > 0.25$). All mice learned the operant response procedures within 3 days of training, showing that D2R overexpression did not impact learning or any phase of the initial training.

D2R overexpression in the NAc, but not the CPu, increases effort for a reward

To test the effect on motivation, we used the PR schedule, which assesses the amount of effort a subject is willing to expend to obtain a reward. We analyzed only two consecutive PR sessions because repeated exposure to PR sessions lowers operant responding over time. Measure of the breakpoint showed a trend towards an increase in the animals overexpressing D2R in the NAc ($t = -1.68$; $P < 0.08$) but not in the CPu ($t = -1.36$; $P = 0.20$) (Supplementary Figure 1). However, since in the current PR schedule the criterion doubled for each successive trial (from 512 to 1024 to 2048 lever presses to obtain the next reward), the number of lever presses was used as a more sensitive continuous primary outcome measure. Independent group comparison revealed that D2R overexpression led to ~50% increase in the number of lever presses in the NAc group ($t = 2.51$; $P = 0.03$) but did not alter total lever presses in the CPu group ($t = 1.66$; $P = 0.12$) (Figures 3a and b).

We next examined the performance of mice in the simple RR tasks and in a lever-pressing/chow-feeding choice procedure in

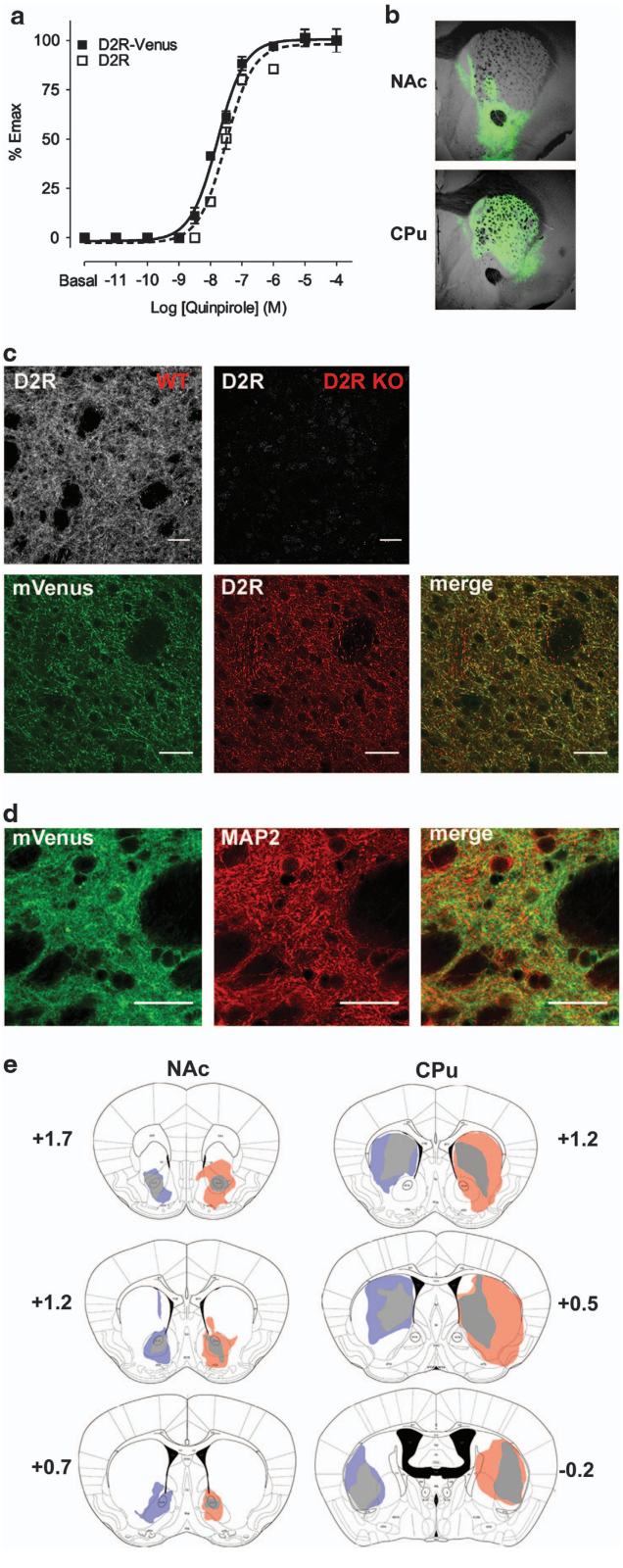

Figure 1. Characterization of the D2R-mVenus overexpression. (**a**) Fusion of mVenus to the D2R receptor did not alter the ability of the agonist quinpirole to activate G protein as demonstrated in an aequorin-based assay. Quinpirole-induced luminescence was determined as described in Materials and Methods and expressed as a percentage of the maximal response for each construct. Results of three independent experiments are represented as mean ± s.e.m. fit to a sigmoidal dose response non-linear regression. (**b**) Representative examples of D2R-mVenus expression by AAV injection in nucleus accumbens (NAc) (top) and dorsal striatum (CPu) (bottom). (**c**) Specificity of the anti-D2R antibody was determined by comparison of a wild-type and D2R knockout (KO) stained sections (top row). D2R-mVenus expression highly overlaps with endogenous D2R expression (bottom row). (**d**) D2R-mVenus colocalized with MAP2, consistent with a dendritic/postsynaptic localization of the receptor. Scale bars: 30 μm. (**e**) Diagrammatic representation of the maximal (colors) and minimal (gray) extent of spread of D2R-mVenus (orange) and GFP (blue) for CPu (right diagram) and NAc (left diagram); numbers indicate distance from Bregma.

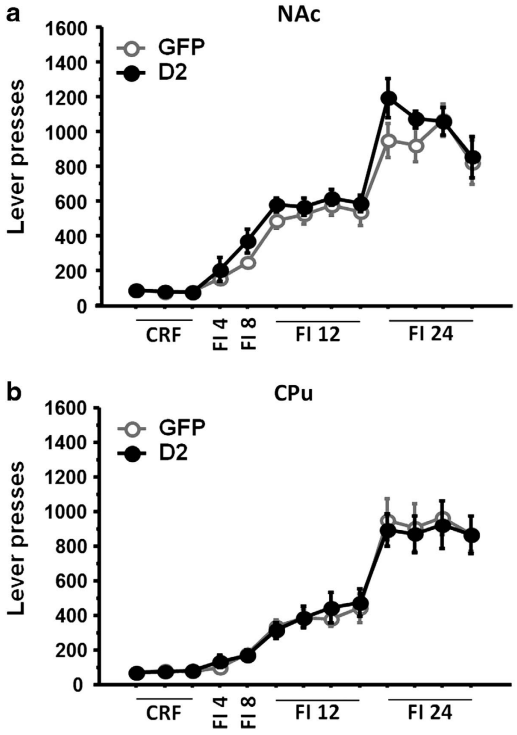

Figure 2. D2R overexpression in the nucleus accumbens (NAc) or the dorsal striatum (CPu) does not alter operant learning. D2R overexpression in the NAc (**a**) or CPu (**b**) did not alter learning of the operant procedure in a continuous reinforcement schedule (CRF) or performance in fixed interval (FI) schedules.

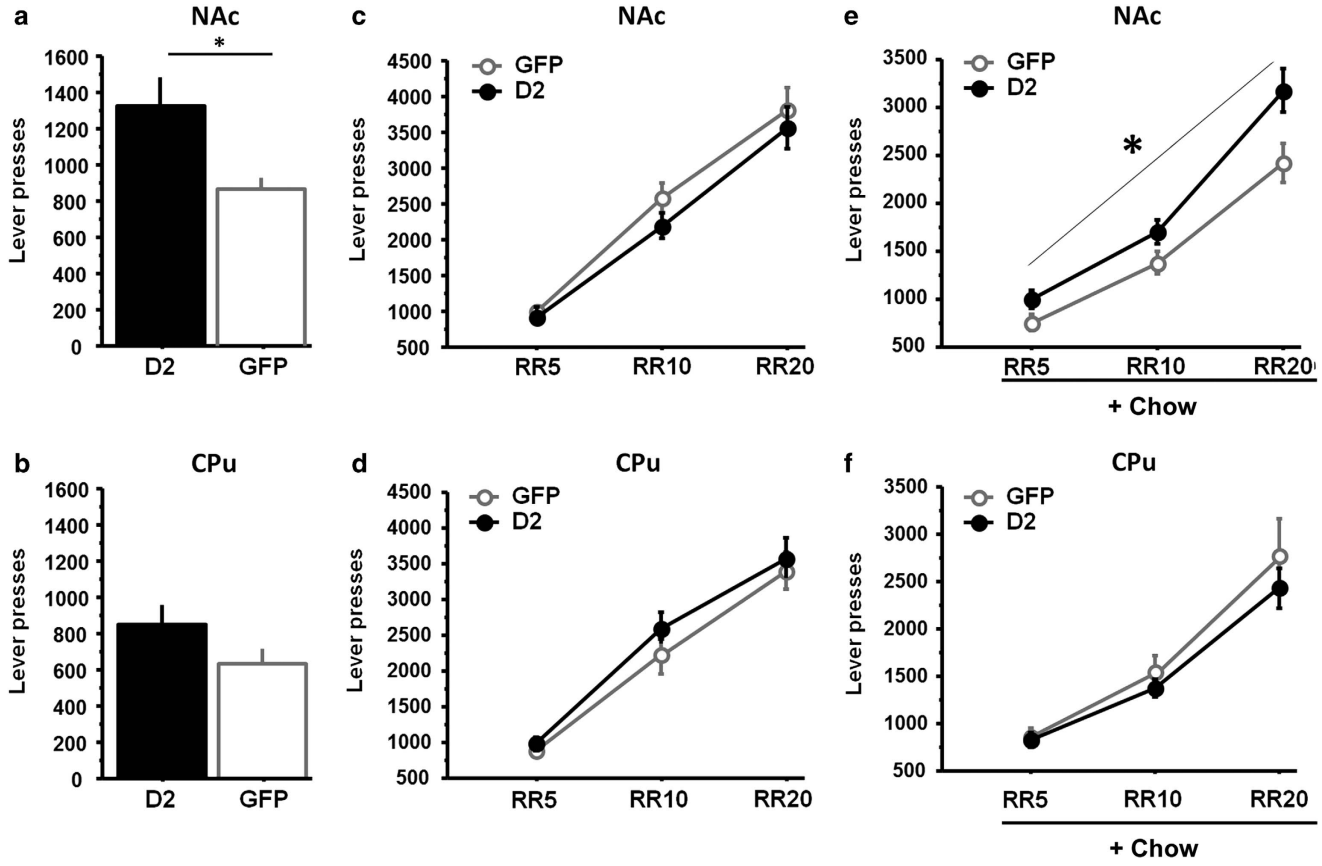

Figure 3. D2R overexpression in the nucleus accumbens (NAc), but not the dorsal striatum (CPu), increases effort in a progressive ratio task and a choice paradigm. D2R overexpression in the NAc (**a**) but not in the CPu (**b**) enhanced the average number of lever presses in a progressive ratio task. When tested in random ratio (RR) paradigms, D2R overexpression in the NAc (**c**) or the CPu (**d**) had no effect on operant response in any of the random ratios tested (number of sessions pooled for each ratios: RR5 = 13; RR10 = 4; RR20 = 7). However, when tested in a choice lever pressing/chow feeding procedure (number of sessions pooled for each ratios: RR5 = 9; RR10 = 8; RR20 = 12), mice overexpressing D2R in the NAc (**e**) showed an enhancement in their rate of lever press, whereas overexpression of D2R in the CPu (**f**) had no effect. *Statistically significant.

which the mice can lever press for preferred food (evaporated milk) or can consume a less preferred food (home cage chow) that is freely available in the chamber.[11] Unlike the PR schedule, which measures willingness to continue working to obtain a reward in the face of an increasing effort requirement, this procedure allows an assessment of the animals' choice to expend effort to obtain a preferred food when a less preferred food is available for little effort. Overall, D2R overexpression had no effect on the number of lever presses emitted on the RR schedule when home cage chow was not available (non-concurrent task), either in the NAc (virus effect: $F_{(1,\ 12)} = 1.25$; $P = 0.28$) (Figure 3c) or the CPu (virus effect: $F_{(1,\ 12)} = 0.65$; $P = 0.44$) (Figure 3d). However, when tested in the choice phase (chow freely available), mice with D2R overexpression in the NAc (Figure 3e) showed enhanced lever presses for the reward in response to the increasing ratio requirements (virus effect: $F_{(1,\ 12)} = 5.51$; $P = 0.04$) and a virus × ratio interaction ($F_{(2,\ 24)} = 4.490$; $P = 0.02$) compared with controls. In contrast, D2R overexpression in the CPu had no effect (virus effect: $F_{(1,\ 12)} = 0.55$; $P = 0.47$; virus × ratio interaction: $F_{(2,\ 24)} = 0.67$; $P = 0.52$) (Figure 3f). The difference in lever presses in the NAc group was directly due to an increase in lever presses in mice overexpressing D2R since the two GFP-expressing control groups were similar ($F_{(1,\ 12)} = 0.68$; $P = 0.43$).

Interestingly, analyses of the ratio (lever presses in choice)/(lever presses in no choice) in the NAc group showed that the D2R-overexpressing animals maintained their rate of lever pressing, whereas GFP-expressing control animals significantly decreased their lever presses when free chow was available in the cage (Figure 4a). ANOVA revealed a virus effect ($F_{(1,\ 12)} = 11.09$; $P < 0.01$), and one-sample analysis revealed that the ratio (lever presses in choice)/(lever presses in no choice) was significantly lower than 1 in the NAc GFP group for each RR (overall analysis: $t = -9.85$; $P < 0.01$; one-sample analysis for each RR: all Ps < 0.01) but not in the NAc D2R group (overall analysis: $t = -1.14$; $P = 0.27$; one-sample analysis for each RR: all Ps > 0.1). In contrast, in the CPu group (Figure 4b) there was no virus effect ($F_{(1,12)} = 2.81$; $P > 0.12$) and the ratios (lever presses in choice)/(lever presses in no choice) were significantly lower than 1 in both groups (overall analysis—CPu GFP: $t = -4.65$, $P < 0.01$; CPuD2R: $t = -6.76$, $P < 0.01$), confirming that the animals decreased their rate of lever presses when chow was available in the cage similar to the NAc GFP group. Mice overexpressing D2R in the NAc (Figure 4c) consumed significantly less free chow than controls at all three ratios (ANOVA: $F_{(1,\ 12)} = 31.39$, $P < 0.01$; *post hoc*: all Ps < 0.01), demonstrating that the increase in willingness to work for the reward was accompanied by a reduction in consumption of freely available, less preferred food.[25] In contrast, D2R overexpression in the CPu group (Figure 4d) had no effect on the amount of chow consumed in the choice phase ($F_{(1,\ 12)} = 1.65$; $P = 0.22$). Altogether, these data demonstrate that D2R overexpression in the NAc enhances the willingness to work for the reward.

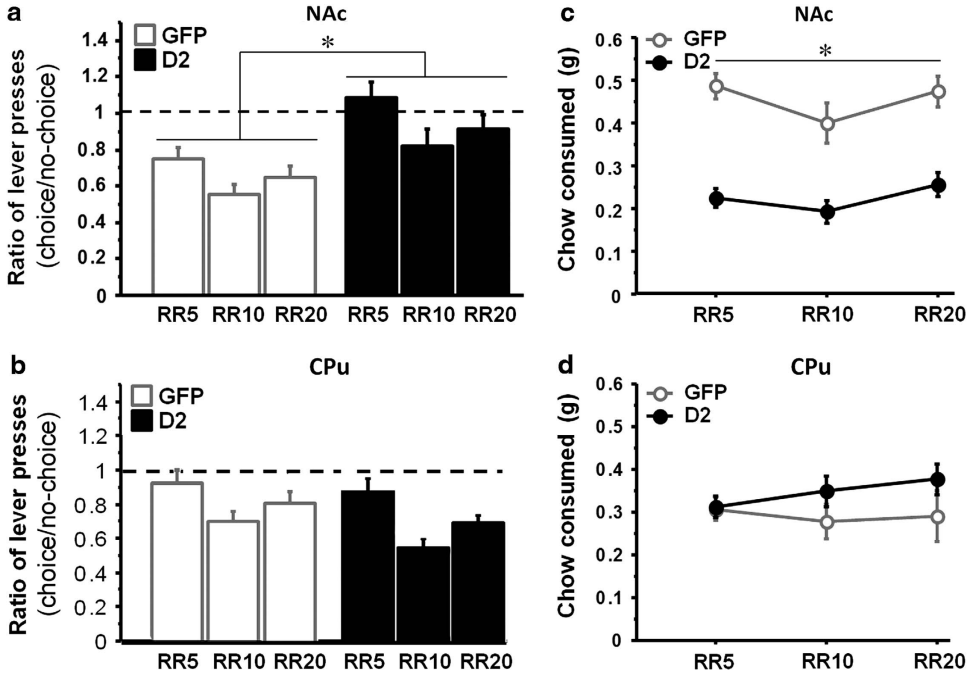

Figure 4. Animals that overexpress D2R in the nucleus accumbens (NAc)—but not the dorsal striatum (CPu)—maintain their rate of lever presses in the choice procedure and eat less of the free chow. (**a**) Ratio (lever presses in choice)/(lever presses in no-choice) in the NAc and CPu (**b**) groups. Amount of chow consumed in the choice paradigm in the NAc (**c**) and CPu (**d**) groups. *Statistically significant.

D2R overexpression does not affect the reactivity to reward, the capacity to represent reward value or the ability of reward-associated cues to alter the motivation to respond

We tested whether the increase in lever pressing in the NAc D2R-overexpressing mice in the reinforcement schedules with high work requirement was related to differences in the capacity to form and update representations of reward value with an outcome devaluation procedure. After subjects have learned that bar pressing leads to a specific outcome (milk), they are allowed to satiate on the reward and then given the opportunity to make the response that previously led to that outcome.[22,26,27]

In the devaluation procedure, free consumption of the evaporated milk reward before testing reduced response rates in all groups significantly more than free consumption of an alternative food, indicating a significant degree of devaluation specific to the reinforcer obtained by bar pressing (Figures 5a and b). ANOVA revealed a significant type of pre-feeding effect on lever pressing in both NAc and CPu groups (both $Ps < 0.01$) but no effect of D2R overexpression in either the NAc ($F_{(1,\ 12)} = 0.67$; $P = 0.43$) or the CPu ($F_{(1,\ 12)} = 1.42$; $P = 0.26$), indicating a similar sensitivity to the current value of outcomes. *Post hoc* comparisons confirmed that all groups experienced outcome devaluation when pre-fed with evaporated milk compared with chow (all *P*-values < 0.03) independent of the overexpression (all *P*-values > 0.13). Similarly, ANOVA revealed a pre-feeding effect on the number of head entries in the food magazine (both $Ps < 0.01$) but no effect of D2R overexpression in either NAc ($F_{(1,\ 12)} = 0.32$; $P = 0.58$) or the CPu ($F_{(1,\ 12)} = 0.92$; $P = 0.36$) (data not shown). There was a significant virus × pre-feeding effect ($F_{(1,\ 12)} = 5.54$; $P = 0.04$) in the NAc group but unpaired comparisons for each pre-feeding did not show any difference (both $Ps > 0.18$), confirming a similar sensitivity to the value of outcomes. There was also no difference in the amount of free evaporated milk consumed by each group during pre-feeding (mean (in g) ± s.e.m.: NAc-GFP, 4.3 ± 0.2; NAc-D2R, 4.7 ± 0.2; CPu-GFP, 4.2 ± 0.4; CPu-D2R, 4.7 ± 0.2; *t*-tests, $Ps > 0.3$), indicating that reactivity to the reward itself was not affected by D2R overexpression.

The ability to learn about cues associated with reward and their capacity to enhance motivation was tested in the PIT—a paradigm in which a neutral stimulus is paired with an unconditioned reward and its ability to modulate motivation to make an instrumental response is tested.[22,26,27] Specifically, PIT was measured by first training the mice to associate a tone-CS with the occurrence of the milk reward. ANOVAs revealed that, overall, both NAc and CPu groups increased their rate of head entries during the tone compared with the ITI (NAc group: $F_{(1,\ 12)} = 13.6$, $P < 0.01$; CPu group: $F_{(1,\ 11)} = 14.6$, $P = 0.01$) and there were no differences based on GFP or D2R overexpression (virus × session effect—NAc group: $F_{(1,\ 12)} = 0.04$, $P = 0.84$; CPu group: $F_{(1,\ 11)} = 3.32$, $P = 0.10$) (Supplementary Figure 2). Thus, the overexpression had no impact on the capacity to learn a CS–US association. The level of PIT was tested in a session in which no primary reward was delivered, but the tone CS was presented intermittently. During this PIT test, mice from both NAc (PIT ratio: $t = -3.43$; $P < 0.01$) and CPu (PIT ratio: $t = -3.31$; $P < 0.01$) groups pressed significantly more during the tone CS than during the ITI (Figures 5c and d). Moreover, the level of PIT (increase in responding during the CS) did not differ between D2R overexpression and control groups in the NAc ($t = -0.41$; $P = 0.69$) or the CPu ($t = -1.92$; $P = 0.09$) (Figures 5c and d), suggesting that the tone was equally effective at modulating motivation in both groups. Similarly, the number of head entries in the food magazine was significantly increased in both groups during the tone presentation compared with ITI (both Fs $< 13,60$; both *Ps* < 0.01), but there was no effect of D2R overexpression (both Fs < 0.65; both *Ps* > 0.44) (data not shown). The similar sensitivities of the mice to reward devaluation and PIT support the idea that the increased motivation in NAc D2R-overexpressing animals is not related to an alteration in reactivity to the reward itself, the representation of reward value or the capacity

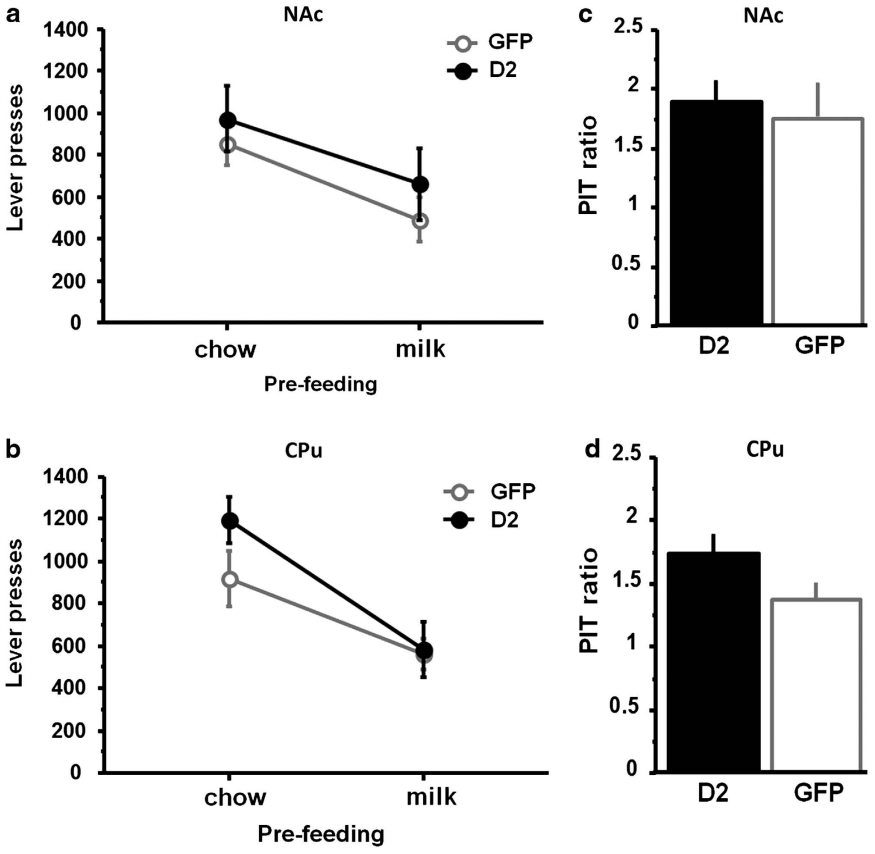

Figure 5. D2R overexpression does not affect representation of outcome or incentive value. D2R overexpression in the nucleus accumbens (NAc) (**a**, **c**) or the dorsal striatum (CPu) (**b**, **d**) had no effect on satiety devaluation (**a**, **b**) by pre-feeding with either chow or evaporated milk, or on Pavlovian-to-instrumental transfer (**c**, **d**).

of stimuli associated with reward to modulate motivation, but rather to a specific enhancement of willingness to work for the reward.

DISCUSSION

Our data show that postsynaptic D2R overexpression in the NAc—but not in the CPu—of adult mice increases motivation. This was shown in two ways: (1) higher levels of operant responding as work requirements increased; and (2) higher levels of responding for the earned reward in the presence of a less preferred but freely consumable reinforcer. The increased motivation was not due to a change in sensitivity to the value of the reinforcer, as the NAc D2R-overexpressing and control animals consumed similar amounts of the food reward when freely available and all groups were similarly sensitive to devaluation of the reward. D2R overexpression also had no effect on acquisition of Pavlovian goal tracking or enhancement of instrumental responding in the presence of an appetitive cue, indicating no change in the Pavlovian components of the instrumental response. Thus, augmenting postsynaptic expression of D2Rs in the NAc in adulthood selectively enhances the ability to sustain the instrumental response—or willingness to work—without changing the animal's representation of the value of the reward stimulus *per se*. Rather, the effect specifically involves an alteration of the representation of how effortful the response is and/or the computation of the difference between cost and benefit.

Imaging studies in humans suggest that optimal goal-directed behaviors and motivation seem to correlate with higher D2R levels in the striatum.[7–9,28] Similarly, high D2R availability in the striatum is associated with a resilience against the development of addiction.[29] Miscalculation of cost–benefit[30–32] as well as decreased D2R availability in the striatum[6,33–35] are common features among human pathologies that involve a dysregulation of motivation. Notably, increasing DA transmission at the D2R in the ventral striatum correlates with improvement in symptoms in attention deficit hyperactivity disorder,[6] and in cocaine abusers, the successful response to a motivation-based treatment is associated with enhanced DA transmission at the D2R in the ventral striatum.[36] Our current findings are consistent with these studies in implicating an important role for ventral striatal D2R signaling in motivation. This may be particularly true for disorders in which a loss of willingness to work is a prominent phenotype.

Our results are in agreement with previous studies showing a critical role for mesolimbic D2R-mediated DA transmission in the regulation of decisions based on effort expenditure.[11] Salamone and co-workers[11,37–41] have shown that DA signaling at the D2R has a powerful effect on an animal's willingness to work for a reward, based on the work-related response costs and the value of the reinforcer itself. Indeed, intra-NAc infusions of low doses of D2R antagonists or DA depletion impair motivation and shift behavior away from food-reinforced tasks that have a high response requirement and toward low-cost options with less reinforcement.[11] Conversely, amphetamine administration in humans[42] or into the NAc in animals facilitates motivation,[43–45] as do manipulations that increase endogenous levels of extracellular DA. Indeed, Cagniard *et al.*[46] showed that knockdown of the DA transporter in mice, which results in elevated extracellular DA as a result of decreased clearance, enhanced the tendency to work for a food reward, without effects

on Pavlovian and operant learning. Similarly, Bello *et al.*[47] showed that mice lacking DA D2 autoreceptors, and thus with elevated striatal DA synthesis and release due to a loss of feedback inhibition, display enhanced motivation to obtain a food reward. It is notable that manipulations that increase extracellular DA levels enhance motivation, and also lead to other behaviors such as impulsivity and/or hyperlocomotion that can interfere with learned appetitive behaviors,[43,48,49] whereas the mice in this study did not display generalized increases in unconditioned behavior (Supplementary Figure 3). These findings are consistent with the above studies showing that blocking D2R signaling impairs motivation and that increasing extracellular DA can enhance motivation, but this study shows that potentiating postsynaptic DA signaling at D2R in the NAc selectively enhances the organism's willingness to work for reward.

These results highlight a potential dissociation between D2Rs in the NAc and CPu in modulating motivation. Recognition that the NAc is involved in goal-directed behavior has led to the hypothesis that mesoaccumbens DA signaling encodes information regarding the motivational significance of a stimulus.[50] Early work showed that neurotoxic lesions of the NAc specifically impair an animal's response to conditioned and unconditioned reinforcers, whereas lesions of the dorsal striatum affect behaviors such as response initiation and reaction time to acquire the reinforcers.[45,51–53] Thus, it has been suggested that instrumental responding for natural rewards is dependent on the NAc, whereas the dorsal striatal pathways recruit stimulus–response processes.[54] Given evidence that the dorsal striatum has a role in instrumental learning, with activity of the nigrostriatal DA pathway acting as a reinforcement signal,[3,55] it is somewhat surprising that D2R overexpression in the CPu did not have a significant effect on any of the tasks performed in this study. Indeed, previous studies demonstrated that restoring DA signaling selectively in the dorsal striatum of DA-depleted mice is sufficient to restore reward-based learning.[56] Anatomical and functional studies suggest that the dorsal striatum is divided into a medial system that supports action–goal associations and a lateral system involved in stimulus–response association.[57] The lack of effect of D2R overexpression in the CPu in this study could result from the concomitant alteration of both instrumental systems. However, this seems unlikely since action of DA in either one of these subregions of the dorsal striatum seems to be able to support instrumental processes independently of DA signaling in the other area.[58] Further studies using overexpression of D2R in specific subregions of the CPu and operant schedules that discriminate between stimulus–response and action–goal associations will be required to understand the role of the D2R in the dorsal striatum for instrumental behavior.

Previous studies have shown that genetically-driven conditional overexpression of D2R in the striatum of mice results in a reversible decrease in motivation.[20,21,24] In those studies, the D2R was overexpressed in mice throughout their development and was associated with a reduction in the operant responding required to earn a reward, opposite to these findings. However, in that transgenic mouse model the decrease in motivation likely reflects an effect of D2R overexpression across development, which results in compensatory alterations in the DA system.[59] Moreover, in that developmental mouse model, D2R overexpression is of a smaller magnitude and is not selective for ventral or dorsal striatum, but is restricted to medium spiny projection neurons throughout the striatum. In the viral model presented here, we specifically targeted the NAc or CPU and produced a large increase in D2R expression in all cells within these regions.

In the striatum, expression of D1R and D2R is largely segregated in neurons of the striatonigral and striatopallidal pathways, respectively,[60] although a subpopulation of MSNs in the NAc coexpress D1R and D2R and may represent a third neuronal pathway.[61] It is possible that NAc D2R overexpression in our study enhances the role of this atypical projection pathway. In addition, cholinergic interneurons in the striatum express D2R[62] and are strongly modulated by reward probability.[63] Thus, the effect on motivation could also be directly related to overexpression of D2Rs in cholinergic interneurons. Ultimately, further studies using targeted manipulation that allows the specific overexpression of D2Rs in discrete neuronal subpopulations in the NAc will be required to determine which neurons are involved in the modulation of motivation. While it is not yet known which neuronal population mediates this effect, our results provide an important proof of concept by showing that increasing postsynaptic D2R density in the NAc selectively enhances motivation by increasing the willingness to expend effort to obtain a goal without major changes in other reward-related processes.

This raises the prospect that therapies that enhance postsynaptic D2R signaling in the NAc could be successful in psychiatric disorders that involve dysregulation of motivation. Along this line, determination of the molecular mechanisms mediating the effect of ventral striatal postsynaptic DA D2Rs on motivation, from the level of gene regulation to specific D2R signaling pathways in neuronal subpopulations, can reveal novel pharmacological targets for motivation enhancement. While a challenging hurdle for pharmacological intervention, development of a functionally selective agonist for postsynaptic D2Rs or specific signaling pathways coupled to these receptors is feasible.[64] Another approach that could selectively enhance postsynaptic D2R expression *in vivo* in the NAc is viral-mediated expression. Of note, adeno-associated viral gene delivery in preclinical studies, in both rodents and non-human primates, has led to promising results in the context of neurodegenerative diseases.[65] Our data support the idea that treatments that increase D2R in specific brain regions might allow the selective modulation of motivation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Claudia Schmauss for the generous gift of D2R KO mice and Christoph Kellendonk for discussion and comments on the manuscript. This work was supported, in part, by US National Institutes of Health Grants DA022413, MH054137 (to JAJ), MH068073 (to PDB), MH068073 (to PDB), MH086404 (to JAJ, HM, EHS), by The Sidney R Baer Jr Foundation (to HM), by a Research Associate Award from the Research Foundation for Mental Hygiene (to PT), an EMBO Long-Term fellowship and the Basque Country Government (to EU), National Institute of Mental Health Grant F32MH090750-01 (to RDW) and by the Lieber Center for Schizophrenia Research and Treatment. PT, JAJ, EHS, PDB and HM designed research; PT, BF, EU and VW performed research; PT, RDW, KMT, PDB and HM analyzed the data; and PT, EHS, PDB, HM, DM and JAJ wrote the paper. All the authors participated in interpreting the data and edited the manuscript.

REFERENCES

1 Koob GF. Hedonic valence, dopamine and motivation. *Mol Psychiatry* 1996; **1**: 186–189.
2 Wise RA. Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox Res* 2008; **14**: 169–183.
3 Schultz W. Behavioral theories and the neurophysiology of reward. *Annu Rev Psychol* 2006; **57**: 87–115.
4 Wang GJ, Volkow ND, Thanos PK, Fowler JS. Imaging of brain dopamine pathways: implications for understanding obesity. *J Addict Med* 2009; **3**: 8–18.
5 Volkow ND, Wang GJ, Fowler JS, Tomasi D, Telang F. Addiction: beyond dopamine reward circuitry. *Proc Natl Acad Sci USA* 2011; **108**: 15037–15042.
6 Volkow ND, Wang GJ, Newcorn JH, Kollins SH, Wigal TL, Telang F *et al.* Motivation deficit in ADHD is associated with dysfunction of the dopamine reward pathway. *Mol Psychiatry* 2011; **16**: 1147–1154.

7 Gjedde A, Kumakura Y, Cumming P, Linnet J, Moller A. Inverted-U-shaped correlation between dopamine receptor availability in striatum and sensation seeking. *Proc Natl Acad Sci USA* 2010; **107**: 3870–3875.
8 Huang CL, Yang YK, Chu CL, Lee IH, Yeh TL, Chen PS *et al.* The association between the Lie scale of the Maudsley personality inventory and striatal dopamine D2/D3 receptor availability of healthy Chinese community subjects. *Eur Psychiatry* 2006; **21**: 62–65.
9 Tomer R, Goldstein RZ, Wang GJ, Wong C, Volkow ND. Incentive motivation is associated with striatal dopamine asymmetry. *Biol Psychol* 2008; **77**: 98–101.
10 Salamone JD, Correa M. The mysterious motivational functions of mesolimbic dopamine. *Neuron* 2012; **76**: 470–485.
11 Salamone JD, Correa M, Farrar A, Mingote SM. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology (Berl)* 2007; **191**: 461–482.
12 Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I *et al.* A selective role for dopamine in stimulus-reward learning. *Nature* 2011; **469**: 53–57.
13 Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)* 2007; **191**: 391–431.
14 Tran AH, Tamura R, Uwano T, Kobayashi T, Katsuki M, Matsumoto G *et al.* Altered accumbens neural response to prediction of reward associated with place in dopamine D2 receptor knockout mice. *Proc Natl Acad Sci USA* 2002; **99**: 8986–8991.
15 Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ. Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science* 2001; **292**: 2499–2501.
16 Hauber W, Sommer S. Prefrontostriatal circuitry regulates effort-related decision making. *Cereb Cortex* 2009; **19**: 2240–2247.
17 Trifilieff P, Rives ML, Urizar E, Piskorowski RA, Vishwasrao HD, Castrillon J *et al.* Detection of antigen interactions *ex vivo* by proximity ligation assay: endogenous dopamine D2-adenosine A2A receptor complexes in the striatum. *Biotechniques* 2011; **51**: 111–118.
18 Han Y, Moreira IS, Urizar E, Weinstein H, Javitch JA. Allosteric communication between protomers of dopamine class A GPCR dimers modulates activation. *Nat Chem Biol* 2009; **5**: 688–695.
19 Jung MY, Skryabin BV, Arai M, Abbondanzo S, Fu D, Brosius J *et al.* Potentiation of the D2 mutant motor phenotype in mice lacking dopamine D2 and D3 receptors. *Neuroscience* 1999; **91**: 911–924.
20 Simpson EH, Kellendonk C, Ward RD, Richards V, Lipatova O, Fairhurst S *et al.* Pharmacologic rescue of motivational deficit in an animal model of the negative symptoms of schizophrenia. *Biol Psychiatry* 2011; **69**: 928–935.
21 Ward RD, Simpson EH, Richards VL, Deo G, Taylor K, Glendinning JI *et al.* Dissociation of hedonic reaction to reward and incentive motivation in an animal model of the negative symptoms of schizophrenia. *Neuropsychopharmacology* 2012; **37**: 1699–1707.
22 Holmes NM, Marchand AR, Coutureau E. Pavlovian to instrumental transfer: a neurobehavioural perspective. *Neurosci Biobehav Rev* 2010; **34**: 1277–1295.
23 Pickel VM, Garzon M, Mengual E. Electron microscopic immunolabeling of transporters and receptors identifies transmitter-specific functional sites envisioned in Cajal's neuron. *Prog Brain Res* 2002; **136**: 145–155.
24 Drew MR, Simpson EH, Kellendonk C, Herzberg WG, Lipatova O, Fairhurst S *et al.* Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. *J Neurosci* 2007; **27**: 7731–7739.
25 Ward RD, Simpson EH, Richards VL, Deo G, Taylor K, Glendinning JI *et al.* Dissociation of hedonic reaction to reward and incentive motivation in an animal model of the negative symptoms of schizophrenia. *Neuropsychopharmacology* 2012; **37**: 1699–1707.
26 Zanich ML, Fowler H. Transfer from Pavlovian appetitive to instrumental appetitive conditioning: signaling versus discrepancy interpretations. *J Exp Psychol Anim Behav Process* 1978; **4**: 37–49.
27 Balleine BW, Ostlund SB. Still at the choice-point: action selection and initiation in instrumental conditioning. *Ann N Y Acad Sci* 2007; **1104**: 147–171.
28 Stelzel C, Basten U, Montag C, Reuter M, Fiebach CJ. Frontostriatal involvement in task switching depends on genetic differences in d2 receptor density. *J Neurosci* 2010; **30**: 14205–14212.
29 Volkow ND, Wang GJ, Fowler JS, Thanos PP, Logan J, Gatley SJ *et al.* Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. *Synapse* 2002; **46**: 79–82.
30 Sonuga-Barke EJ, Fairchild G. Neuroeconomics of attention-deficit/hyperactivity disorder: differential influences of medial, dorsal, and ventral prefrontal brain networks on suboptimal decision making? *Biol Psychiatry* 2012; **72**: 126–133.
31 Monterosso J, Piray P, Luo S. Neuroeconomics and the study of addiction. *Biol Psychiatry* 2012; **72**: 107–112.
32 Rowland NE, Vaughan CH, Mathes CM, Mitra A. Feeding behavior, obesity, and neuroeconomics. *Physiol Behav* 2008; **93**: 97–109.
33 Martinez D, Broft A, Foltin RW, Slifstein M, Hwang DR, Huang Y *et al.* Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. *Neuropsychopharmacology* 2004; **29**: 1190–1202.
34 Nikolaus S, Antke C, Beu M, Muller HW. Cortical GABA, striatal dopamine and midbrain serotonin as the key players in compulsive and anxiety disorders—results from *in vivo* imaging studies. *Rev Neurosci* 2010; **21**: 119–139.
35 Volkow ND, Wang GJ, Telang F, Fowler JS, Thanos PK, Logan J *et al.* Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *NeuroImage* 2008; **42**: 1537–1543.
36 Martinez D, Carpenter KM, Liu F, Slifstein M, Broft A, Friedman AC *et al.* Imaging dopamine transmission in cocaine dependence: link between neurochemistry and response to treatment. *Am J Psychiatry* 2011; **168**: 634–641.
37 Mott AM, Nunes EJ, Collins LE, Port RG, Sink KS, Hockemeyer J *et al.* The adenosine A2A antagonist MSX-3 reverses the effects of the dopamine antagonist haloperidol on effort-related decision making in a T-maze cost/benefit procedure. *Psychopharmacology (Berl)* 2009; **204**: 103–112.
38 Nowend KL, Arizzi M, Carlson BB, Salamone JD. 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* 2001; **69**: 373–382.
39 Pardo M, Lopez-Cruz L, Valverde O, Ledent C, Baqi Y, Muller CE *et al.* Adenosine A2A receptor antagonism and genetic deletion attenuate the effects of dopamine D2 antagonism on effort-based decision making in mice. *Neuropharmacology* 2012; **62**: 2068–2077.
40 Pereira M, Farrar AM, Hockemeyer J, Muller CE, Salamone JD, Morrell JI. Effect of the adenosine A2A receptor antagonist MSX-3 on motivational disruptions of maternal behavior induced by dopamine antagonism in the early postpartum rat. *Psychopharmacology (Berl)* 2011; **213**: 69–79.
41 Sink KS, Vemuri VK, Olszewska T, Makriyannis A, Salamone JD. Cannabinoid CB1 antagonists and dopamine antagonists produce different effects on a task involving response allocation and effort-related choice in food-seeking behavior. *Psychopharmacology (Berl)* 2008; **196**: 565–574.
42 Treadway MT, Buckholtz JW, Cowan RL, Woodward ND, Li R, Ansari MS *et al.* Dopaminergic mechanisms of individual differences in human effort-based decision-making. *J Neurosci* 2012; **32**: 6170–6176.
43 Zhang M, Balmadrid C, Kelley AE. Nucleus accumbens opioid, GABaergic, and dopaminergic modulation of palatable food motivation: contrasting effects revealed by a progressive ratio study in the rat. *Behav Neurosci* 2003; **117**: 202–211.
44 Wirtshafter D, Stratford TR. Evidence for motivational effects elicited by activation of GABA-A or dopamine receptors in the nucleus accumbens shell. *Pharmacol Biochem Behav* 2010; **96**: 342–346.
45 Kelley AE, Delfs JM. Dopamine and conditioned reinforcement. I. Differential effects of amphetamine microinjections into striatal subregions. *Psychopharmacology (Berl)* 1991; **103**: 187–196.
46 Cagniard B, Balsam PD, Brunner D, Zhuang X. Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward. *Neuropsychopharmacology* 2006; **31**: 1362–1370.
47 Bello EP, Mateo Y, Gelman DM, Noain D, Shin JH, Low MJ *et al.* Cocaine supersensitivity and enhanced motivation for reward in mice lacking dopamine D2 autoreceptors. *Nat Neurosci* 2011; **14**: 1033–1038.
48 Yin HH, Zhuang X, Balleine BW. Instrumental learning in hyperdopaminergic mice. *Neurobiol Learn Mem* 2006; **85**: 283–288.
49 Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X. Hyperdopaminergic mutant mice have higher 'wanting' but not 'liking' for sweet rewards. *J Neurosci* 2003; **23**: 9395–9402.
50 Iversen SD, Iversen LL. Dopamine: 50 years in perspective. *Trends Neurosci* 2007; **30**: 188–193.
51 Robbins TW, Roberts DC, Koob GF. 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* 1983; **224**: 662–673.
52 Cador M, Robbins TW, Everitt BJ. Involvement of the amygdala in stimulus-reward associations: interaction with the ventral striatum. *Neuroscience* 1989; **30**: 77–86.
53 Amalric M, Koob GF. Functionally selective neurochemical afferents and efferents of the mesocorticolimbic and nigrostriatal dopamine system. *Prog Brain Res* 1993; **99**: 209–226.
54 Belin D, Jonkman S, Dickinson A, Robbins TW, Everitt BJ. Parallel and interactive learning processes within the basal ganglia: relevance for the understanding of addiction. *Behav Brain Res* 2009; **199**: 89–102.
55 Reynolds JN, Wickens JR. Dopamine-dependent plasticity of corticostriatal synapses. *Neural Netw* 2002; **15**: 507–521.
56 Palmiter RD. Dopamine signaling in the dorsal striatum is essential for motivated behaviors: lessons from dopamine-deficient mice. *Ann N Y Acad Sci* 2008; **1129**: 35–46.

57 Balleine BW, Liljeholm M, Ostlund SB. The integrative function of the basal ganglia in instrumental conditioning. *Behav Brain Res* 2009; **199**: 43–52.
58 Darvas M, Palmiter RD. Restricting dopaminergic signaling to either dorsolateral or medial striatum facilitates cognition. *J Neurosci* 2010; **30**: 1158–1165.
59 Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V *et al.* Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. *Neuron* 2006; **49**: 603–615.
60 Gerfen CR, Surmeier DJ. Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci* 2011; **34**: 441–466.
61 Perreault ML, Hasbi A, O'Dowd BF, George SR. The dopamine d1-d2 receptor heteromer in striatal medium spiny neurons: evidence for a third distinct neuronal pathway in basal ganglia. *Front Neuroanat* 2011; **5**: 31.
62 Alcantara AA, Chen V, Herring BE, Mendenhall JM, Berlanga ML. Localization of dopamine D2 receptors on cholinergic interneurons of the dorsal striatum and nucleus accumbens of the rat. *Brain Res* 2003; **986**: 22–29.
63 Apicella P, Ravel S, Deffains M, Legallet E. The role of striatal tonically active neurons in reward prediction error signaling during instrumental task performance. *J Neurosci* 2011; **31**: 1507–1515.
64 Mottola DM, Kilts JD, Lewis MM, Connery HS, Walker QD, Jones SR *et al.* Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D2 receptors linked to adenylate cyclase. *J Pharmacol Exp Ther* 2002; **301**: 1166–1178.
65 Morgenstern PF, Marongiu R, Musatov SA, Kaplitt MG. Adeno-associated viral gene delivery in neurodegenerative disease. *Methods Mol Biol* 2011; **793**: 443–455.

Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)