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# Dissociation of Hedonic Reaction to Reward and Incentive Motivation in an Animal Model of the Negative Symptoms of Schizophrenia

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We previously showed that mice that selectively and reversibly overexpress striatal D2 receptors (D2R-OE) model the negative symptoms of schizophrenia. Specifically, D2R-OE mice display a deficit in incentive motivation. The present studies investigated the basis for this deficit. First, we assessed whether hedonic reaction to reward is intact in D2R-OE mice. We assessed licking behavior and video-scored positive hedonic facial reactions to increasing concentrations of sucrose in control and D2R-OE mice. We found no difference between D2R-OE mice and controls in hedonic reactions. To further understand the basis of the motivational deficit, mice were given a choice between pressing a lever for access to a preferred reward (evaporated milk) or consuming a freely available less preferred reward (home-cage chow). D2R-OE mice pressed less for the preferred milk and consumed more of the freely available less preferred chow, indicating that striatal overexpression of postsynaptic D2Rs can alter cost/benefit computations, leading to a motivational deficit. This motivational impairment was ameliorated when the transgene was turned off and D2R levels were normalized. Such a deficit may arise from impaired ability to represent the value of future rewards. To test this, we used operant concurrent schedules and found reduced sensitivity to the value of future outcomes in D2R-OE mice. These results demonstrate for the first time in a transgenic animal model of schizophrenia a dissociation between hedonic reaction to reward and incentive motivation, and show a striking parallel to the proposed neurobiological and psychological mechanisms of impaired incentive motivation in schizophrenia.
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## INTRODUCTION

Motivational deficits in schizophrenia are of particular clinical significance, as they greatly impair the overall functioning and quality of life of patients and do not respond well to current medications. Reduced motivation could result from anhedonia, an inability to experience pleasure from events or stimuli that others find pleasurable, or avolition, a deficit in the initiation or maintenance of goal-directed behavior. Surprisingly, given the intuitive relation between hedonia and motivation, recent findings from several research groups indicate a dissociation between hedonic reaction to rewarding stimuli and motivated behavior in patients with schizophrenia (Cohen and Minor, 2010; Gard *et al*, 2007; Heerey and Gold, 2007). Although there are counterexamples (Strauss *et al*, 2011), the majority of the current literature shows relatively intact subjective hedonic reaction to rewarding stimuli, but impaired incentive motivation.

Although the molecular underpinnings of impaired incentive motivation in schizophrenia are not precisely known, animal research points to distinct neurobiological substrates underlying volition and hedonia. Dopamine (DA) signaling has been shown to be critical for incentive motivation (Salamone *et al*, 2007), but is relatively uninvolved in hedonic reaction to reward (Berridge and Robinson, 2003). Thus, to the extent that altered DA signaling is an important part of the pathophysiology of schizophrenia (Davis *et al*, 1991), one might anticipate changed incentive motivation but unaltered hedonic

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reactions. In addition, mounting evidence suggests that one function of DA D2 receptor (D2R) signaling in motivated behavior is to affect the cost/benefit computation that determines the willingness to expend a given effort to obtain a particular reward (Salamone *et al*, 2007). Thus, the altered D2R activity observed in patients (Abi-Dargham *et al*, 2000) might be expected to have a specific effect on this aspect of motivation.

To assess the role of increased activity of striatal D2Rs in the pathogenesis of schizophrenia-relevant neurobiological and behavioral phenotypes, Kellendonk *et al* (2006) generated mice that selectively overexpress D2Rs (15% increase) in striatal medium spiny neurons (D2R-OE). We previously showed that these mice exhibit motivational impairments. Specifically, D2R-OE mice quit working sooner than controls on progressive-ratio schedules, in which the number of lever presses required for food reward increases following each reward (Drew *et al*, 2007). This impairment is not due to increased sensitivity to satiety or fatigue, or to differential sensitivity to other features of the progressive-ratio schedule (Simpson *et al*, 2011).

Although results from previous experiments indicate that D2R-OE mice exhibit a deficit in motivation, the specific reasons for this deficit remain unknown. Therefore, the purpose of the current experiments was to identify the specific nature of the reduced motivation in D2R-OE mice. First, we assessed hedonic reaction in D2R-OE mice and found that like patients, hedonic reaction to reward was intact in D2R-OE mice. We then found that the decreased motivation arose from decreased willingness to expend effort to obtain reward. Furthermore, we found that D2R-OE mice are less sensitive to the relative value of positive outcomes than controls. The decreased willingness to work may therefore be the result of a deficit in the representation of future outcomes and/or an imbalance in the cost/benefit computation. These factors may also be a critical component of the incentive motivation deficit in patients with schizophrenia.

## SUBJECTS AND METHODS

### Subjects

The generation of D2R-OE mice, temporal regulation of the transgene, and food restriction protocol have been previously described (Drew *et al*, 2007; Simpson *et al*, 2011; Kellendonk *et al*, 2006; Ward *et al*, 2009) and are detailed in the Supplementary Materials.

### Experimental Procedures

The apparati used in the present experiments are detailed in the Supplementary Methods.

*Gustometer testing.* A total of 8 control and 8 D2R-OE mice were used. Initial gustometer training is described in the Supplementary Methods. For testing, each mouse was subjected to two 30-min test sessions. The procedure for running the test sessions was similar to that used during training sessions 2 and 3 (eg, 5-s trials and 7.5-s intertrial intervals). During a test session, the mouse was offered six sipper tubes: one contained water and the other five each contained a different concentration of sucrose (0.03, 0.1, 0.3, 0.6, and 1.0 M). We treated the six solutions as a block, and randomized (without replacement) the order of presentation of each solution within a block so that each solution was presented once before the initiation of a second block. The mouse could initiate up to 48 blocks. To motivate the mice to initiate a large number of trials during the 30-min test session, we restricted them from food and water for 23 h before each test session. Each mouse was provided with a single 1 g chow pellet (F0173, Bio-Serv; Frenchtown, NJ) and 2 ml of tap water; this amounted to $\sim$19% and 30% of their normal daily food and water intake, respectively (J Glendinning, unpublished data). We ran each mouse through two test sessions. We presented the same sucrose concentrations during each test session. We gave each mouse 1 day of *ad libitum* food and water between test sessions.

We converted each mouse's licking response to each sucrose concentration into a standardized lick ratio (SLR) to control for potential individual and strain differences in local lick rate. First, local lick rate was calculated from responses during training day 1, when the mice had unlimited access to water. For each mouse we computed a mean interlick interval (ILI). We excluded ILIs $>200$ ms as they are thought to reflect pauses between bursts of licking. The reciprocal of the mean ILI was used as the local lick rate. Using this method, we found that the mean ($\pm$ SE) local lick rate (in licks/s) was 8.7 ($\pm$ 0.1) for WT mice and 8.8 ($\pm$0.2) for D2R-OE mice. To calculate the SLR, we divided the rate of licking for a given taste stimulus (across both test sessions) by the baseline local lick rate. In this way, each mouse's reaction to different sucrose concentrations is normalized against its own baseline lick rate.

*Taste-reactivity testing.* A total of 7 control and 8 D2R-OE mice were used. We used the taste-reactivity paradigm developed by Grill and Norgren (1978) to measure hedonic reactions to increasing concentrations of sucrose. A voluntary sucrose drinking procedure was used, similar to that used in other experiments (Berridge, 2000; Pecina *et al*, 2003). These experiments were conducted in a transparent testing chamber. A mirror positioned below the floor of the chamber reflected a view of the mouse's face and mouth into the close-up lens of a video camera to enable videotaping of affective reactions. All mice were pre-exposed to the testing chamber for 30 min per day for 5 sessions before the testing phase. During these sessions, a 1-ml drop of 1.0 M sucrose was placed on the floor of the chamber to accustom the mice to drinking sucrose presented in this way.

Following pre-exposure, the mice were given daily sessions which consisted of exposure to one of four different sucrose concentrations (0.01, 0.1, 0.3, and 1.0 M). Over the course of testing, each mouse was exposed to each concentration of sucrose twice. Presentation order for the different concentrations of sucrose throughout sessions was randomized such that each mouse had a different presentation order and experienced each concentration once before repeating any concentration. The mice were continuously recorded throughout the session. Independent raters blind to the genotypes of the mice viewed frame-by-frame videotapes and scored them for the number of positive reactions for each mouse at each sucrose concentration, number of bouts (defined as a series of licks without pause

for $>2$ s), and the number of positive reactions per bout. Positive reactions scored were rhythmic tongue movements, lateral tongue protrusions, rhythmic mouth movements, and paw licking (Berridge, 2000; Pecina *et al*, 2003, Pecina *et al*, 1997). Behavior was scored for 5 min, beginning with the first lick. Inter-rater reliability was assessed by computing the percentage agreement between two raters from a randomly selected 15 sessions. There was over 90% agreement on all behavioral measures.

*Preference assessment.* A total of 8 control and 10 D2R-OE mice were used. This experiment was conducted to empirically validate that evaporated milk was preferred to home-cage chow. Mice received two 1-h sessions separated by 3 days with no testing. In one session, consumption of evaporated milk *vs* home-cage chow was compared. In a second session, consumption of water *vs* home-cage chow was compared. The experiment took place with two cohorts of mice. In the first cohort, control ($n=5$) and D2R-OE mice ($n=3$) were first exposed to the milk *vs* chow session, followed by the water *vs* chow session. In the second cohort (control, $n=3$ and D2R-OE, $n=7$), the order of session presentation was counterbalanced across mice. Preference assessments were conducted in standard holding cages.

*Operant procedures.* Four groups of mice were used (control $n=8$, control-Dox $n=8$, D2R-OE $n=7$, and D2R-OE-Dox $n=7$). Mice were trained to consume the liquid reward and to lever press exactly as previously described (Simpson *et al*, 2011; see Supplementary Materials).

*Effort-related choice procedure.* The mice were tested in the effort-related choice paradigm, an operant assay of incentive motivation. Unlike the progressive-ratio schedules we used before, which assess willingness to continue working for a reward as the response requirement increases, this procedure assesses willingness to expend effort to obtain a preferred reward *vs* consuming a freely available less-preferred reward (see, eg, Salamone *et al*, 1991). Thus, this procedure allows us to assess the relative value of working for a preferred reward when a less preferred reward is available for no effort. Mice were first placed on a random ratio (RR)-5 schedule, in which the number of lever presses per reward varied randomly from reward to reward but the average number of lever presses per reward was five. After several sessions of RR-5, a dish of home-cage chow was introduced during the session. The RR schedule value was then increased to RR 10, 15, and 20 over the course of several sessions. Sessions lasted for 60 min. The number of responses was recorded, and the home-cage chow was weighed before and after the session (including any spillage) to determine the amount of chow consumed.

*Matching in concurrent schedules.* A total of 12 control and 12 D2R-OE mice were used in this experiment. All mice had been trained to press levers as described above and had equivalent experience with an unrelated operant procedure before the beginning of the current experiment. In the present experiment, the mice were exposed to concurrent schedules in which rewards were arranged for left and right lever presses according to separate variable interval (VI) schedules in which there is a variable period of time from one reward until the next one becomes available. To discourage mice from strict alternation between levers, a 2-s changeover delay (Catania, 1966) was in effect such that a response following a changeover from one lever to the other could not be reinforced for 2 s. VI schedules were arranged such that there was a fivefold difference in the frequency of rewards obtained from each lever. In one condition, the schedule of reward delivery on the left and right levers was VI 20 s and VI 120 s, respectively, whereas in another condition, the VI schedule values associated with the left and right levers were reversed. Each mouse was exposed to a particular lever-schedule pairing for 10 sessions, after which the schedule values were reversed. The order of exposure to the two sets of concurrent-schedule values was counterbalanced across mice. Data from the last three sessions of exposure to each set of concurrent schedules were pooled and used for data analysis.

Results were analyzed using ANOVA with appropriate terms or independent samples *t*-tests. When appropriate, *post hoc* comparisons were analyzed using Bonferonni tests. All statistical comparisons were conducted with a significance level of $P<0.05$.

## RESULTS

### D2R Overexpression Does Not Alter Reactivity to Sucrose

To obtain a measure of behavioral reaction to a palatable substance, we used a gustometer to record licking behavior as a function of sucrose concentration (see, eg, Glendinning *et al*, 2002). In previous studies, both mice and rats show increased licking as sucrose concentration increases, and this is thought to reflect the palatability of the sucrose solution (Glendinning *et al*, 2002; Spector *et al*, 1993). Figure 1 shows that under the lowest concentration of sucrose, lick ratios obtained from both control and D2R-OE mice were around 0.20, indicating that the mice were licking at a rate of around 20% of their baseline licking rate. Lick ratios for both genotypes increased with increasing concentration ($F(4,56)=76.70$), so that at the higher sucrose concentrations, the lick ratio for both genotypes was around 80% of their baseline rate (mean licks per trial

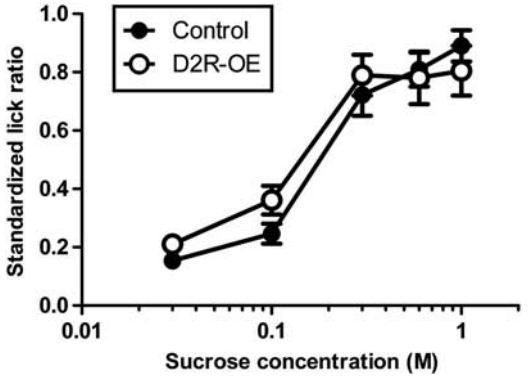


**Figure 1** Concentration response curves showing the standardized lick ratio (see Subjects and Methods for calculation) obtained for control (closed circles) and D2R-OE (open circles) mice as a function of increasing sucrose concentration. Note the *x*-axis is on a log scale.

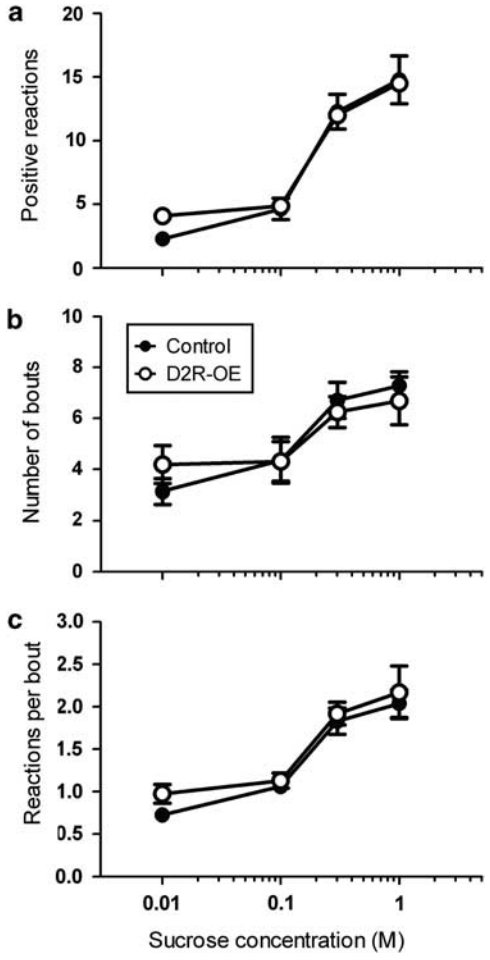


**Figure 2** Hedonic response to increasing sucrose concentration in control (closed circles) and D2R-OE (open circles) mice. (a) Mean number of positive hedonic reactions. (b) Mean number of bouts of drinking. (c) Mean number of positive hedonic reactions per bout. Note the *x*-axis is on a log scale.

for both genotypes are presented in the Supplementary Materials). There was no genotype difference in lick ratios, and no interaction between concentration and genotype. In addition, there was no difference in the number of trials initiated ($t(14) = 1.30$) between control and D2R-OE mice. These data show that the vigor and sensitivity of consummatory behavior to changes in palatability is not altered in D2R-OE mice.

### D2R Overexpression Does Not Alter Hedonic Reaction to Sucrose

Palatable foods have been shown to elicit homologous patterns of facial affective reactions in humans, non-human primates, and rodents (Berridge, 2000). Therefore, by measuring the facial reactions to substances that differ in palatability, hedonic reaction may be quantitatively assessed in mice. We tested the D2R-OE mice in the taste-reactivity paradigm (see, eg, Grill and Norgren, 1978; Pecina *et al*, 1997; Pecina and Berridge, 2005), using sucrose as the palatable food. For all mice, only positive hedonic reactions were observed during the test sessions. No negative reactions such as mouth gapes, head-shakes, face washes,

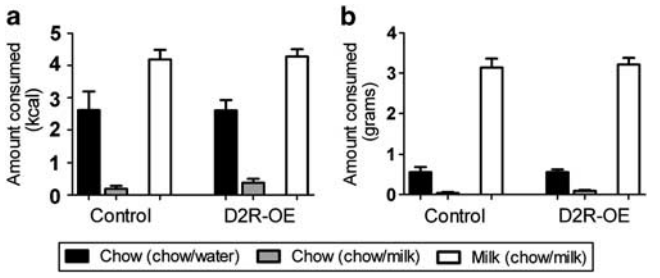


**Figure 3** Results of a preference test showing the amount of home-cage chow consumed by control and D2R-OE mice in the presence of evaporated milk and water. Consumption was calculated both in terms of kcal (a) and grams (b). Also shown is the amount of evaporated milk that was consumed during the preference test.

or paw flails occurred. Figure 2a shows that both control and D2R-OE mice exhibited concentration-dependent increases in the number of positive hedonic reactions in response to sucrose ($F(3, 39) = 68.11$), with the number of reactions increasing from around 5 to around 15 during the 5-min observation period. Similarly, both control and D2R-OE mice initiated more bouts of licking (Figure 2b) ($F(3, 39) = 18.38$), and more positive reactions per bout (Figure 2c) ($F(3, 39) = 34.33$) as sucrose concentration increased. There was no significant effect of genotype or interaction effects in any of these measures. It is clear from these data that both control and D2R-OE mice exhibit equivalent positive hedonic reactions to sucrose.

### Evaporated Milk Is a Highly Preferred Reward for Both D2R-OE and Control Mice

Given that D2R-OE mice show normal consummatory behavior and hedonic reactions, yet perform poorly on progressive-ratio schedules (Drew *et al*, 2007; Simpson *et al*, 2011), we assessed whether D2R-OE mice expressed normal preference for evaporated milk before using it as the reward in our assessment of willingness to work for a preferred outcome (see, eg, Salamone *et al*, 1991). Figure 3 shows the results of a preference assessment where foods were freely available. Because the evaporated milk and chow differ in the number of calories per gram (g), the data are presented as both kcal (Figure 3a) and g (Figure 3b) consumed. Regardless of the metric used to assess chow consumption, both control and D2R-OE mice consumed significantly less chow when given a choice between chow and evaporated milk than when given a choice between chow and water ($F(1, 16) = 50.86$ and 50.98, for the main effect of choice condition on kcal and g of chow consumed, respectively). There was no difference between genotypes and no interaction between choice condition and genotype. In addition, when both chow and milk were available at the same time, all mice consumed significantly more milk than chow ($t$'s $> 12.00$). We conclude from these data that evaporated milk is a preferred reward for both control and D2R-OE mice.

### Striatal D2R Overexpression Produces Decreased Willingness to Work for a Preferred Reward That Is Rescued by Normalization of D2R Levels

Next, we tested the D2R-OE mice in the effort-related choice paradigm, an operant paradigm that indexes incentive

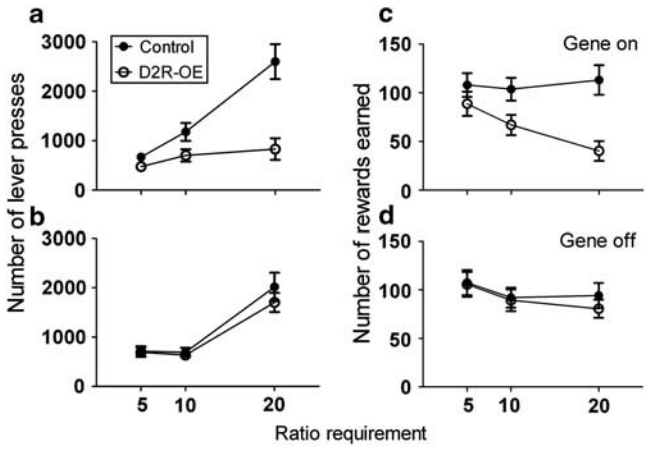


**Figure 4** The right panels show the number of lever presses as a function of ratio requirement during the effort-related choice paradigm in control (closed circles) and D2R-OE (open circles) mice when the transgene was on (a) and when it was turned off by feeding the mice doxycycline (b). The left panels show the number of rewards earned at each ratio requirement for control and D2R-OE mice when the transgene was turned on (c) and when it was turned off (d).

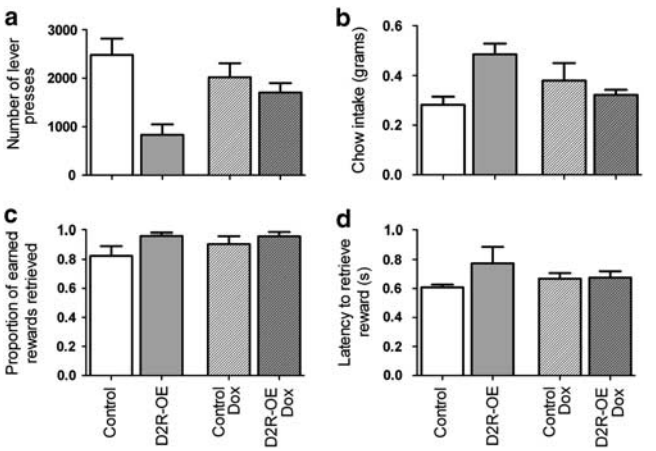


**Figure 5** Results from the effort-related choice paradigm in control and D2R-OE mice. Also shown are the results from two separate groups of control and D2R-OE mice that were fed doxycycline. (a) Mean number of lever presses emitted to obtain the preferred reward of evaporated milk. (b) Mean grams of freely available less preferred chow consumed. (c) Mean proportion of earned rewards that were retrieved. (d) Mean latency to retrieve milk rewards once they were earned.

motivation and is dependent on normal D2R signaling (Farrar *et al*, 2010; Salamone *et al*, 2007). In this procedure, mice could earn evaporated milk by pressing a lever or could consume freely available home-cage chow that was placed in a dish on the floor of the test chamber. Mice were trained to respond on RR schedules, in which the average ratio requirement increased from 5 to 20 across sessions. We first assessed the effect of the ratio requirement on the total amount of work the mice were willing to expend to obtain the evaporated milk reward. For this analysis, data were averaged across all sessions of the RR-5 and RR-10 ratio schedules and across the last 5 sessions of the RR-20 schedule. Figure 4a shows that at the lowest ratio requirement, the number of lever presses emitted by control and D2R-OE mice was similar. As the ratio requirement increased, the number of lever presses emitted by control mice also increased, but responding of D2R-OE mice did not ($F(2, 26) = 8.75$, for the interaction between genotype and ratio requirement). The *post hoc* Bonferroni comparisons found that the number of lever presses between control and D2R-OE mice did not differ significantly at schedule values of RR-5 ($t(13) = 0.68$) and RR-10 ($t(13) = 1.67$), but there was a significant difference in the number of responses between genotypes at RR-20 ($t(13) = 6.19$). Figure 4b shows that this difference between control and D2R-OE mice was eliminated when the transgene was turned off by feeding the mice Dox, indicated by a significant main effect of ratio requirement ($F(2, 26) = 80.36$), but no significant interactions.

Next, we compared the number of rewards earned at each RR schedule value by control and D2R-OE mice. Figure 4c shows that as the ratio requirement increased the number of rewards earned by control mice stayed relatively constant, whereas the number of rewards earned by D2R-OE mice decreased ($F(2, 26) = 5.14$, for the interaction between genotype and ratio requirement). Similar to the lever pressing data, *post hoc* Bonferroni comparisons found no significant differences in the number of rewards earned between genotypes at schedule values of RR-5 ($t(13) = 1.10$) and RR-10 ($t(13) = 2.11$), but the difference was significant at RR-20 ($t(13) = 4.18$). Figure 4d shows that this difference between control and D2R-OE mice was eliminated when the transgene was turned off, evidenced by the lack of a significant interaction between ratio requirement and genotype, although the effect of ratio requirement was significant ($F(2, 26) = 8.51$).

Performance on the effort-related choice procedure is depicted in Figure 5. Figure 5a shows the number of lever presses emitted by the four groups of mice averaged across the last five sessions on the RR-20 schedule, whereas Figure 5b shows the amount of home-cage chow consumed during the session. D2R-OE mice worked substantially less than control mice for the preferred reward of evaporated milk, but consumed substantially more home-cage chow than control mice. A two-factor (genotype × Dox) ANOVA conducted on the lever press data in Figure 5a found a significant effect of genotype ($F(1, 26) = 12.80$), but not of Dox, with a significant interaction ($F(1, 26) = 5.88$). *Post hoc* Bonferroni comparisons found a significant difference in the number of lever presses emitted by control and D2R-OE mice when the transgene was turned on ($t(13) = 4.25$) but no difference when the transgene was turned off by feeding the mice Dox ($t(13) = 0.81$). Similar analyses conducted on the amount of home-cage chow eaten (Figure 5b) found no overall effect of genotype ($F(1, 26) = 2.53$), but a significant interaction between genotype and Dox ($F(1, 26) = 7.51$). As with the lever pressing data, *post hoc* Bonferroni comparisons found a significant difference in the amount of home-cage chow consumed by control and D2R-OE mice when the transgene was turned on ($t(13) = 3.02$) but not when it was turned off ($t(13) = 0.85$).

Within this experiment, we also evaluated responsiveness to reward by measuring the number of earned rewards that were retrieved as well as the latency to retrieve them.

Figure 5c shows the proportion of earned rewards that were retrieved by all groups of mice. All mice retrieved the majority of rewards they earned. There was a tendency for D2R-OE mice (both on and off Dox) to retrieve more earned rewards than controls, but this did not reach statistical significance ($F(1, 26) = 3.82$, for the main effect of genotype). In addition, there was no effect of Dox treatment and no interaction between genotype and Dox treatment. Thus, D2R-OE mice were not less reactive to rewards than controls.

Figure 5d shows the average latency to retrieve earned rewards for all groups of mice. Although the average latency to retrieve rewards appears greater for D2R-OE mice than controls, this is not significant because the mean is distorted by a single mouse in the D2R-OE group that had an average latency of almost twice the group mean. Statistical analysis revealed no significant differences between genotypes and no effect of Dox treatment, with no interaction ($Fs < 2.00$).

### D2R-OE Mice Are Less Sensitive to Differences in Reward Value Than Controls

One important aspect of willingness to work is accurately representing the value of future outcomes. Although D2R-OE mice might show as strong a preference as controls for milk *vs* home-cage chow when the two outcomes are freely available, when they are working for milk they may be less sensitive to the difference in relative value between the two outcomes. To assess whether D2R-OE mice and controls differed in their sensitivity to reward value, we tested control and D2R-OE mice in concurrent schedules with a fivefold variation in reward frequency (VI 20 VI 120: VI 120 VI 20). Typically, in concurrent schedules, the ratio of responses to the two alternatives ($B_1/B_2$) approximates or 'matches' (Herrnstein, 1961) the ratio of rewards obtained from the two alternatives ($R_1/R_2$) according to the following equation:

$$\log(B_1/B_2) = a\log(R_1/R_2) + \log b \quad (1)$$

where $a$ is a parameter that reflects sensitivity to changes in the reward ratio, and $\log b$ is the bias toward one response option over the other (Baum, 1974). The obtained response and reward data (see Supplementary Materials) from each mouse from each pair of concurrent schedules were fit with this equation, yielding estimates of sensitivity and bias for all subjects. Figure 6 shows that the values of the sensitivity parameter obtained from Equation (1) were positive for both control and D2R-OE mice, indicating that response allocation was sensitive to changes in the distribution of rewards across the two alternatives. D2R-OE mice, however, were significantly less sensitive to variations in the distribution of rewards than controls ($t(22) = 2.53$). Estimates of bias were negligible (bias parameter for control and D2R-OE mice was $-0.04$ and $-0.001$, respectively) and did not differ between groups ($t(22) = 0.34$). These results indicate that D2R-OE mice are less sensitive to the value of response options associated with different frequencies of reward than controls.

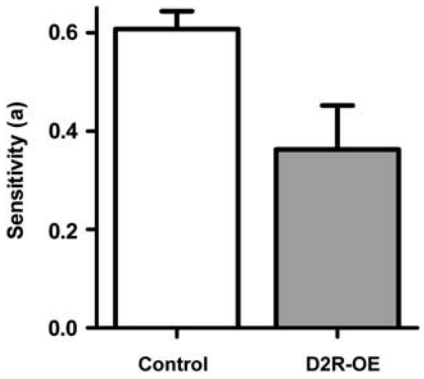


**Figure 6** Mean value of the sensitivity parameter ($a$) estimated from fitting the concurrent-schedule data from control and D2R-OE mice with Equation 1 (see Results for details).

## DISCUSSION

### D2R Overexpression Does Not Impact Consummatory Behavior or Hedonic Reaction to Reward

The results from the gustometer test and the taste-reactivity paradigm are similar to those reported previously in mice, both in terms of the magnitude and level of increase in the standardized lick ratio with increasing sucrose concentration in the gustometer test (see, eg, Glendinning *et al*, 2002) and also in the number of positive hedonic reactions displayed with increasing sucrose concentration during the 5-min taste-reactivity test sessions (Cagniard and Murphy, 2009; Pecina *et al*, 2003). Together, these results indicate that hedonic reaction to appetitive stimuli is not compromised in D2R-OE mice.

In addition to the results from the taste-reactivity paradigm and the gustometer test, several other results from both previous and the present experiments suggest that D2R-OE mice do not differ from controls in their reaction to reward. First, D2R-OE mice displayed preference equivalent to controls for evaporated milk over home-cage chow. In addition, there was no difference between control and D2R-OE mice in the number of earned rewards that were retrieved or the latency to retrieve them (described here and by Simpson *et al*, 2011). In sum, all of the evidence we have point to the conclusion that striatal D2R overexpression does not alter hedonic reactivity to the presentation of appetitive rewards.

The fact that D2R overexpression leaves hedonic reaction to reward intact is consistent with the affective neuroscience literature that indicates that DAergic systems are not heavily involved in hedonic reactions (Berridge, 1996, 2009; Berridge and Robinson, 2003; Berridge *et al*, 2009). For example, in rats, administration of DAergic agents had no effect on hedonic reaction to sucrose (Pecina *et al*, 1997; Treit and Berridge, 1990). Perhaps most striking, depletion of DA via 6-hydroxydopamine lesions of either the nigrostriatal (Berridge *et al*, 1989) or both the nigrostriatal and mesolimbic DA neurons (Berridge and Robinson, 1998) also had no effect on hedonic reaction to sucrose. Similarly, DA-deficient mice exhibited robust consumption of earned food rewards in an appetitive T-maze paradigm, suggestive of intact hedonic reaction to reward (Robinson *et al*, 2005).

Finally, knockdown of the DA transporter, which elevates synaptic DA levels by 70%, had no effect on hedonic reaction to sucrose (Pecina *et al*, 2003). Thus, the present results in combination with the prior studies make a very strong case that DA signaling is not a primary determinant of hedonic reactions.

### D2R Overexpression Produces a Deficit in Willingness to Work for Reward

D2R overexpression produced a deficit in incentive motivation in the effort-related choice paradigm. This deficit is consistent with a large literature on the critical role of DAergic signaling in motivated behavior in a number of paradigms (see Salamone *et al*, 2007; Salamone *et al*, 2009, for reviews). In the effort-related choice paradigm, administration of DA antagonists (either systemically or targeted locally to the nucleus accumbens) decreases lever pressing and increases chow intake, as does depletion of accumbens DA (see, eg, Cousins and Salamone, 1994; Farrar *et al*, 2010; Salamone *et al*, 1991; Salamone *et al*, 1996; Salamone and Correa, 2002). The present results provide further evidence that normal striatal DA signaling is crucial to motivated behavior in this paradigm.

Interestingly, overexpression of striatal D2 receptors produces a motivational phenotype that is similar to the effects of acute D2 antagonism. One possible explanation for this phenomenon is that incentive motivation depends critically on an optimal level of D2 activity, with too much or too little resulting in decreased incentive motivation. Alternatively, chronic overexpression of D2 receptors may lead to reversible downstream changes in intracellular signaling pathways that are similar to changes under acute antagonism of these receptors, thus producing a similar effort-related choice phenotype (see Drew *et al*, 2007 for discussion and other alternatives). Another alternate possibility is that overexpression of striatal D2Rs leads to lower DA activity through a negative feedback mechanism. We did not find any difference in DA content or DA turnover in striatal tissue from D2R-OE and control mice to support such a mechanism (Simpson EH, Kellendonk C, Moore, Kandel ER, unpublished data). However, *in vivo* measurement of extracellular striatal DA could potentially reveal a decrease in DA tone in D2R-OE mice.

It is also important to note that a number of findings indicate that different substructures within the striatum mediate different aspects of incentive motivation (Yin *et al*, 2008). Because overexpression of D2Rs is not differentiated across different areas of the striatum in our model, the potential differential contributions of overexpression of D2Rs in ventral *vs* dorsal striatum to the incentive motivational deficits reported here cannot be determined.

The willingness to work for a preferred reward is dependent on a cost/benefit computation in which the organism must weigh the potential benefits of obtaining the reward with the costs, in terms of energy and time expended, of procuring it (van den Bos *et al*, 2006; Salamone *et al*, 2007). Nucleus accumbens D2R transmission is an important part of the forebrain circuitry that is associated with adaptive cost/benefit computations of work-related response costs. In addition, accumbens D2R manipulations impact instrumental responding (ie, work-related response costs) only when the instrumental requirements become sufficiently arduous (Salamone *et al*, 2007), suggesting a distortion in the cost/benefit computation that disproportionately affects willingness to expend effort when work requirements are relatively difficult. Consistent with these results, when the work required to obtain reward was low (eg, RR-5 and RR-10) D2R-OE and controls did not differ in the amount of effort they were willing to expend to obtain the reward, but when the work requirement became more substantial (RR-20), D2R-OE mice were less willing to work than controls. This result is also consistent with our previous results that showed that the willingness to continue working on the progressive-ratio schedule varied with the difficulty of the work requirement (Simpson *et al*, 2011). Importantly, the modulation of effort by ratio requirement makes alternative interpretations of the performance deficit based on other aspects of the procedure, such as decreased tolerance for delayed rewards in D2R-OE mice, less plausible. Thus, the available evidence suggests that the cost/benefit computation is different in D2R-OE and controls. This difference could arise because D2R overexpression increases the anticipated cost of work or because of an attenuated difference in the future value of milk *vs* the value of chow that is immediately freely available, particularly when the work requirement for milk becomes more difficult. In the current studies we provide evidence that one likely contributor to the computational difference is that D2R overexpression impairs sensitivity to the relative value of response options associated with future rewards. The results of the concurrent-schedule assessment indicate that D2R-OE mice are less sensitive to changes in the distribution of rewards in this paradigm than controls. This impaired sensitivity is consistent with a deficit in the ability to either represent or update the value of response outcomes with changing contingencies. Future experiments could test this possibility in other paradigms that measure sensitivity to the value of future rewards, such as delay discounting. In this paradigm, mice are given the opportunity to choose between a smaller reward delivered immediately and a larger reward delivered after a delay (see, eg, Mazur, 1987). Reduced sensitivity to the value of future rewards in D2R-OE mice would be suggested by a stronger preference for the smaller immediate rewards compared with controls. In addition, although our previous data indicate that D2R-OE mice are not less tolerant of delayed rewards than controls in a progressive-delay paradigm (see Simpson *et al*, 2011; Ward *et al*, 2011), this paradigm will further test whether decreased tolerance for delayed rewards plays a role in the motivational deficit in D2R-OE mice.

In terms of possible circuit-level mechanisms underlying the present results, a large body of work has documented the importance of the prefrontal cortex (PFC) in behavioral and cognitive flexibility. We have previously reported that striatal D2R overexpression produces disturbances in PFC function in D2R-OE mice (Kellendonk *et al*, 2006). Specifically, striatal D2R overexpression results in increased levels of DA, decreased DA turnover, and increased D1 receptor activation in PFC. In addition, more recent results suggest that striatal D2R overexpression may produce a deficit in inhibitory transmission in the PFC, leading to a state of cortical hyperactivity (Li *et al*, 2011). Given the critical

importance of the balance of cortical excitatory and inhibitory transmission for information processing and cognitive flexibility (Yizhar *et al*, 2011), including sensitivity to the value of response options associated with future rewards (Gruber *et al*, 2010), this imbalance may play a critical role in the behavioral deficits reported here.

### The Deficit in Effort-Related Choice Is Reversible

The fact that the deficit in incentive motivation in the effort-related choice paradigm was rescued when the transgenic D2Rs were turned off indicates that the motivational deficit is a result of the effects of acute overexpression of D2Rs, rather than irreversible changes in neural structure or function that result from developmental overexpression. The most obvious change in D2R-OE mice that occurs when the transgene is switched off is that D2R expression levels are normalized. Thus, perhaps returning striatal D2R signaling to normal levels is responsible for the motivational rescue. However, as mentioned above, D2R antagonists themselves decrease the willingness to work for reward. Even with low chronic doses of haloperidol we were unable to reverse the motivational deficit (Simpson *et al*, 2011). This is concordant with the clinical finding that D2R antagonism does not treat the motivational deficits in patients (Manschreck and Boshes, 2007). Exploring new targets for modulating these circuits thus seems a promising way to discover new treatment strategies (see Simpson *et al*, 2011).

### Relevance to Schizophrenia

The present results are relevant to understanding the neurobiological and psychological mechanisms of impaired incentive motivation in schizophrenia, as our animal model captures some of the key behavioral characteristics in patients. First, the present study has shown for the first time in a transgenic animal model of schizophrenia, a dissociation between hedonic reaction to reward and incentive motivation. These results parallel those found in patients (Strauss *et al*, 2011), and suggest that overactivity of striatal D2Rs, as found in patients, does not impact hedonic reaction to reward, but is sufficient to produce the deficit in incentive motivation by decreasing willingness to work. We suggest that decreased willingness to work in D2R-OE mice arises from deficits in the ability to represent or update the value of positive outcomes as has recently been described in patients. A number of studies have reported evidence for impaired representation of the value of positive outcomes, or in using this information to effectively and adaptively guide goal-directed behavior in patients (Barch and Dowd, 2010; Gold *et al*, 2008). In particular, patients appear severely impaired in situations that require relative value judgments (Strauss *et al*, 2011), as in the effort-related choice procedure and the concurrent schedules used in the current studies. The present profile of behavioral results in D2R-OE mice is consistent with an interpretation of impaired relative value judgments. We further suggest that impaired ability to represent or update the value of positive outcomes leads to an imbalance in the cost/benefit computation associated with goal-directed behaviors by either (1) exaggerating the anticipated cost and/or (2) diminishing the anticipated benefit, and that motivational impairments in schizophrenia could result from this imbalance. Finally, and perhaps most importantly, to the extent that D2R-OE mice model the effects of developmental overactivity of striatal D2Rs in patients, the fact that the motivational deficit was reversible, and therefore due to the acute (rather than to the developmental) effects of D2R overexpression, suggests that motivational impairments in patients should be responsive to therapeutic interventions that restore D2R signaling to normal levels.

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## DISCLOSURE

The authors declare no conflict of interest.

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