# Examining the role of Dopamine D2 Receptors in cost-benefit decision making processes underlying motivated behavior in mice

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## *Contributions*

Following the initial discovery of the Progressive Hold Down task as a novel method for assessing goal-directed behavior by graduate student Matthew R. Bailey and Dr. Greg Jensen (Bailey et al., 2015), the current research project was conceived by Matthew R. Bailey but jointly designed by Elke S. Schipani, Dr. Peter D. Balsam, and Matthew R. Bailey. Behavioral assays were done in collaboration between Elke S. Schipani, laboratory technician Eileen Chun, and Matthew R. Bailey. All statistical analyses were done independently by Elke S. Schipani. Data and results were interpreted by Elke S. Schipani with assistance and guidance from both Matthew R. Bailey and Dr. Peter D. Balsam.

*Abstract*

Motivation is a process critical for the survival of organisms, directing and invigorating behavior. Recent human and animal studies have revealed that processing information about costs and benefits is important to adaptive goal-directed behavior, suggesting that cost-benefit decision making plays a crucial role in influencing motivated behavior. Current behavioral tasks which assay cost-benefit decision making often measure willingness to expend effort, but also manipulate reward value simultaneously. To study the distinct roles of effort and value, we develop two tasks in which we offered subjects either a choice between different types of work or a choice between different reward values. By giving subjects a choice between types of work, bar pressing or bar holding, or value, pellets and sucrose concentration, and parametrically altering the relative effort between them, the Concurrent Effort Choice (CEC) and Concurrent Value Choice (CVC) task create functions of choice behavior which represent the calculation of effort or value, respectively. Using pharmacological and genetic manipulations of the Dopamine D2 receptor (D2R), which has specifically been shown to be critically involved in dopamine's modulation of motivated behavior, we address the hypothesis that D2R signaling affects the assessment of effort while leaving value representation unaltered. We first examine the effects of acute dopamine D2 receptor antagonism on cost-benefit decision making using these novel behavioral assays. We further characterize the role of the D2 receptor by examining a genetic model which overexpresses the D2 receptor specifically within the striatum.

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# Abbreviations

| | |
|---|---|
| CEC | Concurrent Effort Choice Task |
| CVC | Concurrent Value Choice Task |
| DA | Dopamine |
| D2R | Dopamine D2 Receptor |
| D2R-OE | Striatal Dopamine D2 Receptor Overexpressing Mice |
| EBC | Effort-Based Choice Task |
| FH | Fixed Hold |
| FR | Fixed Ratio |
| HAL | Haloperidol, Dopamine D2R Antagonist |
| ITI | Intertrial Interval |
| PR | Progressive Ratio Task |
| RR | Random Ratio |
| VEH | Vehicle |
| VIH | Variable Interval Hold |

## *Introduction*

Motivation is a process critical for the survival of organisms, directing and invigorating behavior towards goals allowing them to obtain resources from the environment like food, water, and shelter. Examples of this adaptive behavior reveal the various processes influencing motivation. Animals foraging for food in the wild must integrate information about the relative effort and expected value of future action by comparing the anticipated energy required to the value of food resulting from exploring the environment. At a given time, one of the many possible comparisons of expected effort and value is involved in an integrative process which influences the animal's motivation to forage. Indeed, human and animal studies have revealed that processing of cost and benefit are important to adaptive goal-directed behavior, suggesting that cost-benefit decision-making plays a crucial role in influencing motivated behavior (Salamone et al., 2007). Furthermore, the relationship between cost and benefit varies depending on the situation and the individual organism's needs; thus, examining the distinct neurobiological mechanisms underlying cost-benefit decision-making is important for understanding the basic mechanisms of motivation.

Over the course of behavioral motivation research, several assays have attempted to parse apart processes underlying goal-directed behavior in various ways. The earliest behavioral assays designed to assess motivation in animals employed operant lever pressing tasks with work requirements (i.e., schedules of reinforcement). One such task is the progressive ratio (PR) task, which requires an incrementally increasing number of lever presses to earn each subsequent reward, and measures motivation by level of responding (Hodos, 1961). Through these tasks, it was found that the willingness to work is distinct from the immediate experience of pleasurable stimuli (Berridge & Robinson, 1998). This has been confirmed by neurobiological research

showing that separate neural circuits and neurotransmitters control the willingness to work for a reward than those for hedonic reactions to a pleasurable outcome. Dopamine receptor antagonists and depletion of dopamine in the Nucleus Accumbens have been found to decrease an animal's willingness to emit responses across various behavioral tasks, suggesting that the mesolimbic dopamine system modulates the willingness to work (Salamone et al., 2007). Alternatively, it has been found that opioid receptors involved in the limbic circuit are critical for experiencing pleasure in response to a rewarding stimulus (Smith and Berridge, 2007).

More recent research has begun to analyze the role of effort in motivated behavior by employing choice between high and low effort alternatives, such as the operant effort-based choice (EBC) and the T-maze barrier tasks. The EBC task offers the animal a choice between working for a preferred reward by lever pressing to obtain rewards or making a low effort choice of consuming freely available, less preferred, chow (Salamone et al., 2007). The T-maze barrier task also gives subjects a choice; however, this is between choosing a high effort option of climbing over a physical barrier to get to an arm of the T-maze which has a larger reward (i.e., 4 pellets) or select the other arm without a barrier containing a smaller reward (i.e. 1 pellet) (Salamone et al., 1994; Mott et al., 2009). In both the EBC and the T-Maze barrier task, the proportion of selections made for the high-effort alternative is used to measure a subject's effort-based decision-making.

It was found through these choice tasks that dopamine is critically involved in effort-based decision-making between two effort options. In the EBC task, dopamine antagonists or dopamine depletions decrease willingness to work for the more highly preferred reward, as subjects make fewer lever presses and consume more freely available chow (Salamone et al., 1991, 2002; Koch et al. 2000; Nowend et al. 2001; Sink et al. 2008; Farrar et al. 2010). In the T-

maze barrier task, systemic treatment with dopamine D1 and D2 antagonists decrease the likelihood of choosing the high-effort/high-reward arm (Bardgett et al., 2009; Salamone et al. 1994; Cousins et al. 1996; Mott et al. 2009; Mai et al. 2012; Pardo et al., 2012), whereas increasing dopamine levels with systemic treatment of amphetamine increase the likelihood of choosing the high-effort/high-reward arm (Bardgett et al., 2009).

Though tasks offering a choice between high- and low-effort alternatives further distinguish processes involved in goal-directed behavior, whether the same neural circuits process the experiencing of pleasurable stimuli and expected value cannot be elucidated by these tasks. In both of these tasks, effort and value are manipulated but not completely dissociated, as high effort is always associated with high reward, while low effort is associated with low reward (Gold et al., 2015). Furthermore, these choice tasks fail to use the generalized matching law, which analyzes whether an animal exhibits a bias, a preference for a particular type of work or reward that cannot be accounted for by reinforcement alone, or sensitivity, the amount of change in behavior with change in reinforcement (Baum 1974; Reed and Kaplan, 2011). Because of this, it is unclear whether dopamine modulates bias or sensitivity to effort requirements. Finally, while dopamine manipulations are known to leave in-the-moment hedonic reactions to positive rewards unaltered, these tasks cannot definitively rule out the role of dopamine in bias and sensitivity to reward values. Therefore, we designed a set of tasks that either maintain effort or value constant while parametrically varying the other in order to parse apart the effect of each on goal-directed behavior (Gold et al., 2015).

The current work also aims to extend previous research into the role of the dopamine D2 receptors (D2R) in cost-benefit decision-making, which has specifically been shown to be

involved in dopamine's modulation of motivated behavior through both pharmacological and genetic manipulations of the D2R.

Pharmacological antagonism of the D2R has been shown to decrease responding in progressive ratio tasks in a dose-dependent manner (Aberman et al., 1998) and significantly reduces lever pressing for a preferred reward in the EBC task while increasing the amount of less-preferred, freely available chow consumed (Salamone et al., 1991).

A manipulation of the D2 receptor in a genetic model leads to motivation deficits similar to those seen with acute antagonism of the receptor, however, the mechanism by which the D2 receptors is manipulated is entirely different. Transgenic mice which selectively overexpress the cloned human dopamine D2Rs in the striatum (D2R-OE) were generated such that the expression of this transgene can be reversed by a regimen of doxycycline (DOX), normalizing D2R expression levels (Kellendonk et al., 2006). These D2R-OE mice show deficits in incentive motivation as observed through deficits in responding on PR (Drew et al., 2007; Simpson et al., 2011), as well as in an EBC task where D2R-OE mice pressed less for milk reward and consumed more lab chow (Ward et al., 2012). These impairments have been shown to be reversed through the normalization of D2R overexpression following treatment with DOX (Drew et al., 2007; Simpson et al., 2011; Ward et al., 2012). It has been shown that the observed motivational deficits are not due to altered appetite or feeding, and that the hedonic reaction remains intact (Ward et al., 2012).

The results of the pharmacological and genetic manipulations of the D2 receptor implicate this receptor in motivated behavior, but which specific processes the D2R modulates has yet to be fully understood. It has been suggested that in effort-based decision making, D2R signaling affects the assessment of effort while leaving value representation unaltered (Salamone

et al., 1991; Gold et al., 2015). On the other hand, others have suggested that D2R signaling modulates value representations (Horvitz et al., 1988). In the present experiments, we explicitly assessed the effect of D2R in the calculation of effort and value to address this hypothesis by specifically examining the role of the D2R in the calculation of both effort and value when making choices among goal-directed actions. We develop two tasks termed the Concurrent Effort Choice (CEC) and the Concurrent Value Choice (CVC) tasks, in which we offered the animal either a choice between different types of work in the CEC task or a choice between different reward values in the CVC task. We first established the validity of the paradigms, then examined the effects of acute D2 receptor antagonism, followed by an assessment of chronic D2R overexpression. By parametrically altering the effort and value in these two tasks, we specifically address the hypothesis of the D2 receptors role in cost-benefit decision-making.

## *Materials and Methods*

### Animals

A total of 32 adult male C57BL6/J drug-naïve mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used to optimize outcomes at baseline as well as for testing pharmacological manipulations of the dopamine D2R in experiments examining effort (n = 16) and value (n = 16). All subjects were housed on 12h light/dark cycles and maintained on chow (Isopro RMH 3000 complete mouse diet).

A total of 58 adult female mice were used for two experimental cohorts, one used to study effort (n=30), the other value (n=28). Mice were either D2R-OE transgenic mice created as described previously (Kellendonk et al., 2006), which have the Dopamine D2 receptors (D2R) overexpressed specifically within the striatum, or C57BL/6J:129SvEvTac F1 hybrid control littermates which express normal levels of D2R. Subjects were maintained on either regular home cage chow (Isopro RMH 3000 complete mouse diet), or DOX-supplemented chow (Prolab, Syracuse, NY) yielding 4 experimental groups: D2R-OE animals on DOX (effort n=8; value n=6) and off DOX (effort n=8; value n=6), and control animals on DOX (effort n=8; value n=9) and off DOX (effort n=6; value n=6).

Throughout the experiment mice were food-restricted to 85% of baseline weight to motivate them to work for food reinforcers. Water was provided *ad libitum*. All animal use was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by New York State Psychiatric Institute and Columbia University Animal Care and Use committees.

**Drug Treatments and Dose Selection**

The Dopamine D2 receptor antagonist Haloperidol (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.2% lactic acid solution was injected intraperitoneally 45 minutes prior to behavioral testing. 0.2% lactic acid solution was used as a vehicle control. Doses used were 0.1 and 0.2 mg/kg for the effort experiment and 0.1 mg/kg for the value experiment and were based on previous research (Salamone et al, 1991, 1994, 2001, 2002, 2009).

**Apparatus**

Experimental chambers were used as described previously (Drew et al., 2007). Briefly, each chamber contained two retractable levers, a food hopper where mice could earn a liquid reward of 0.01mL, a pellet dispenser which delivered a sucrose pellet reward, a food trough able to detect head entries, a house light, and a speaker, which delivered a tone when the dipper rose.

## *Behavioral Procedures*

### Operant Training

Mice were trained to lever press as has been described previously (Drew et al., 2007). Briefly, subjects in the effort cohorts first learned to receive milk rewards from the dipper inside a food trough, while subjects in the value cohorts were trained to receive rewards of 20% sucrose solution from the dipper or a sucrose pellet from the pellet dispenser. Mice next learned to press a lever to earn a reward, and were reinforced for every lever press they made.

### Ratio Training

For random ratio training, reinforcers could be earned with multiple presses of a lever at predetermined ratios. Mice were first trained on an RR-05 schedule where the mean ratio requirement was 5 presses. A variable intertrial interval (ITI) where the lever was retracted and the house light turned off followed each reinforcer. The house light and the extension of the lever indicated the beginning of a new trial. Each session lasted until the mouse had earned 60 reinforcers or 1hr had elapsed. The mice were run on each random ratio schedule (RR05, RR10, and RR20) for 2 days.

### Hold-Down Training

Mice in the effort choice cohorts were trained on Variable Interval Hold (VIH) schedule of reinforcement, which reinforced lever presses held for a randomly determined amount of time in each trial (Bailey et al., 2015). Intertrial intervals occurred after each reinforcer earned as described in the RR schedule. Each session ended after the mouse had earned 40 reinforcers or 1 hour had elapsed. Mice were run on various VIH schedules (0.5, 1, 2, 4, 6, 8, and 10 s) before

moving on to the PHD task. In PHD, the mice were required to hold the lever for a duration that increased in length after each subsequent reinforcer. The session ended after 2 hours had elapsed. The last successful hold made in a session defined its break point.

**Concurrent Effort Choice Training**

Mice were trained for CEC on a task that used both levers, one that represented a 5 second hold requirement, and the other that represented a fixed ratio (FR) 5 requirement. The training task extended one lever per trial, and required the mouse to make the correct ratio requirement according to the lever extended in order to earn a reinforcer. The lever side was counterbalanced with respect to the work requirement. A variable ITI averaging 120 seconds where the lever was retracted and the house light turned off followed each reinforcer. Each session ended after the mouse had earned 40 reinforcers or 1 hour had elapsed. The training task was run for three consecutive days before the mice moved on to the Concurrent Effort Choice (CEC) task. The CEC task began with ten forced choice trials, where one lever was extended at a time, indicating the fixed hold or ratio requirement for the session. The lever was retracted after 3 minutes extended without the subject completing the work requirement Following the forced trials were choice trials where both levers were extended with their given fixed hold and ratio requirements. The lever on which the mouse had been trained to press or hold was maintained throughout these sessions. A variable ITI averaging 120 seconds followed each reinforcer. Each session ended after the mouse had earned all reinforcers for the session or 1 hour had elapsed. Mice were first run on schedules with a 5 second hold requirement and 1, 5, 10, 20, 40, and 80 ratio requirements, and then schedules with a 10 second hold requirement and 1, 5, 10, 20, 40,

80, and 160 ratio requirements. Each hold and ratio requirement combination was run at least twice in ascending ratio requirement order.

**Concurrent Value Choice Training**

Mice were trained for CVC on a task that employed both levers, both employing ratio requirements, but one that delivered a sucrose pellet reward, the other a sucrose solution dipper reward. The training task extended one lever per trial, and required the mouse to make the correct ratio requirement according to the lever extended in order to earn a reinforcer. A variable ITI where the lever was retracted and the house light turned off followed each reinforcer. Each session ended after the mouse had earned 40 reinforcers or 1 hour had elapsed. The training task was run for three consecutive days before the mice moved on to the Concurrent Value Choice task. The CVC task began with ten forced choice trials, where one lever extended at a time indicating the ratio requirement of each lever for the session. Following the forced trials were choice trials where both levers extended with their given fixed ratio requirements. The lever on which the mouse had been trained to receive sucrose solution or pellets was maintained throughout these sessions. A variable ITI averaging 120 seconds followed each reinforcer. Each session ended after the mouse had earned all reinforcers or 1 hour had elapsed. Mice were first tested using a 20% sucrose solution for reward after five presses on the dipper lever, or a sucrose pellet after the completion of a fixed ratio requirement on the pellet lever (FR05, 10, 20, 40, 80). Mice were then run using the same ratio combinations with a 5% sucrose solution. Each fixed ratio requirement combination was run at least twice.

## *Experimental Procedures*

### Experiment 1a: Examining the relationship between two types of effort, holding and pressing for a reward

To assess the ability of the CEC task to relate two types of work, mice were trained to make lever presses and lever holds of separate levers and were tested in 2 baseline phases: fixed 5 second hold (FH05) with varying ratio requirements over days (1, 5, 10, 20, 40, 80, 160) and fixed 10 second hold (FH10) with varying ratio requirements over days (1, 5, 10, 20, 40, 80, 160).

### Experiment 1b: Examining the relationship between reward types, sucrose pellets and sucrose solutions

To assess the ability of the CVC task to relate two rewards, mice were trained to press for liquid sucrose and sucrose pellets on separate levers. Subjects were tested in 2 baseline phases: 1) fixed ratio 5 requirement (FR05) for 20% sucrose solution vs. varying ratio requirements for sucrose pellets (10, 20, 40, 80); 2) FR05 for 5% sucrose solution vs. varying ratio requirements for sucrose pellets (10, 20, 40, 80).

### Experiment 2a: Effects of the Dopamine D2R antagonist haloperidol on Calculation of effort.

To assess the effects of Haloperidol in the CEC task the same mice which were used in experiment 1a then repeated the procedure in 1a to establish baseline functions for subjects' sensitivity to different effort requirements. In a next drug phase, mice were tested in the fixed 10 second hold condition at ratio requirements of 10, 20, 40, and 80. Subjects were injected with Vehicle, 0.1 mg/kg Haloperidol, and 0.2 mg/kg Haloperidol 45 minutes prior to behavioral

testing in a randomized Latin Squares design across the different schedules such that each animal was tested on each schedule on a given dose twice.

**Experiment 2b: Effects of the Dopamine D2R antagonist haloperidol on Calculation of value.**

To assess the effects of Haloperidol in the CVC task the same mice which were used in experiment 1b then repeated the procedure in 1b but were injected with Vehicle, 0.1, and 0.2 mg/kg Haloperidol 45 minutes prior to behavioral testing in a randomized Latin Squares design across the different schedules such that each animal was tested on each schedule on a given dose twice.

**Experiment 3a: Effects of striatal Dopamine D2R overexpression on Calculation of effort.**

To assess the effects of D2 receptor overexpression in the striatum on effort sensitivity in the CEC task mice were trained to make lever presses and lever holds of opposite levers.

The 4 groups, control on DOX (n=8), control off DOX (n=6), D2R-OE on DOX (n=8;), and D2R-OE off DOX (n=8) were then tested in 3 different CEC Hold Duration Phases: 1) FH05 with varying ratio requirements over days (1, 5, 10, 20, 40, 80, 160) 2) FH10 with varying ratio requirements over days (1, 5, 10, 20, 40, 80, 160), and 3) fixed 20 second hold (FH20) with varying ratio requirements over days (1, 5, 10, 20, 40, 80, 160).

**Experiment 3b: Effects of striatal Dopamine D2R overexpression on Calculation of value.**

To assess the effects of D2 receptor overexpression in the striatum on value sensitivity in the CVC task mice were trained to press for liquid sucrose and sucrose pellets on opposite levers. The 4 groups, control on DOX (n=8), control off DOX (n=8), D2R-OE on DOX (n=6;), and D2R-OE off DOX (n=6) were then tested in 2 different CVC Sucrose Value Phases: 1) FR05 for

20% sucrose solution vs. varying ratio requirements for sucrose pellets (5, 10, 20, 40, 80); 2) FR05 for 5% sucrose solution vs. varying ratio requirements for sucrose pellets (5, 10, 20, 40, 80).

### *Statistical Analyses*

Baseline CEC and CVC data were analyzed using repeated measures two-way ANOVA. Significant group effects were then subjected to *post hoc* analysis using paired *t* tests. Data for the pharmacological manipulations of the CEC and CVC task were subjected to both repeated measures two-way and three-way ANOVAs. Significant group effects were then subjected to *post hoc* analysis using paired *t* tests. Data for the genetic manipulations of the CEC and CVC were analyzed using between-subject ANOVA. Significant group effects were then subjected to *post hoc* analysis using unpaired *t* tests. ANOVA results are reported in the text, while *post hoc* findings are designated in figures with asterisks. Detailed *post hoc* analysis can be found in Appendix A. A Bonferroni correction was applied to all measures. $\alpha$ was set equal to 0.05.

## *Results*

### Effort Manipulations Alter Choice Behavior When Choosing Between Two Alternative Types of Work

To examine effort based choice behavior, the CEC task gives subjects a choice between 2 types of work (bar pressing or bar holding). Increasing the ratio requirements on the ratio lever across sessions while keeping the hold requirement constant resulted in an increase in the proportion of rewards earned by completing the hold requirement, shown by a sharp increase in the proportion of rewards earned holding after FR20 for both 5 and 10 second hold requirements (Fig 1A). As Figure 1A indicates, the proportion of hold choices was influenced by both the hold requirements and the press requirements. Analyzing the proportion of hold choices across the two hold requirements, five seconds and ten seconds, revealed a significant main effect of ratio requirement ($F_{(6, 90)} = 163.0$; $p < 0.0001$), a significant main effect of hold requirement ($F_{(1, 15)} = 88.75$; $p < 0.0001$), and a significant ratio by hold interaction ($F_{(6, 90)} = 3.553$; $p = 0.0033$). To further analyze the choice behavior, we extracted a point of subjective equality (PSE) for each subject, which estimates the number of presses that would yield an equal chance of the subject employing the ratio or hold bar to complete the trial. The PSE was modulated by the manipulation of hold duration (Fig 1B), as there was a significant difference in the baseline PSEs of FH05 and FH10 requirements ($t_{(15)} = 5.143$; $p < 0.0001$) where a ten second hold requirement requires a greater hypothetical ratio requirement to make rewards earned by completing the requirement on each bar approximately equal. Thus, increasing the hold requirement alters behavior such that as the ratio requirement increases, subjects are less willing to opt for the hold alternative.

Further analysis of choice behavior revealed that the number of responses made on the ratio bar changed as a function of ratio requirement. Presses increased as ratio requirements increased up until FR20, at which point the number of responses made on the press bar began to decrease, as subjects became more likely to employ the hold bar. There was a significant effect of the ratio requirement ($F_{(6, 90)} = 17.76$; $p < 0.0001$) the hold requirement ($F_{(1, 15)} = 30.98$; $p < 0.0001$) a significant ratio by hold requirement interaction affecting this variable ($F_{(6, 90)} = 4.376$; $p = 0.0006$; Fig 1C). This reflects an increase in proportion of rewards earned employing the hold lever after the FR20 ratio requirement. Interestingly, the number of presses made by subjects when the hold alternative was 5 seconds appears to drop off from the FR20 requirement more dramatically than when the fixed hold requirement was 10 seconds. This reflects differences we observe in the PSE between hold requirements where subjects continue working on the ratio lever to earn reinforcers at higher requirements when the alternative is a 10-second hold.

When subjects do choose to hold, their hold efficiency, the number of successful holds made over the total holds, increases as a function of ratio requirement across both hold requirements. Understandably, when the fixed hold requirement is 5 seconds, subjects are more efficient, completing hold requirements more successfully than when they are twice as long. There was a significant main effect of ratio requirement ($F_{(6, 90)} = 16.17$; $p < 0.0001$), hold requirement ($F_{(1, 15)} = 57.44$; $p < 0.0001$), and no significant hold by ratio requirement interaction ($F_{(6, 90)} = 1.447$; $p = 0.2058$; Fig 1D).

Because we varied the effort requirements over days, subjects were exposed to the press requirement and hold duration of a given day in the first 10 trials of a session so they were aware of these effort levels prior to making choices between the two types of work. In these first 10

forced trials, subjects were exposed to 5 trials with the hold lever and 5 trials with the press lever. If a subject stopped working for 3 minutes in any of the ten forced trials they were designated as an opt out, and the subject moved on to the next trial. The number of opt outs gave an initial indication of the effectiveness of manipulating effort requirement on the press lever. Overall, there were very few opt outs over the course of the experiment. It was only when ratio requirements became particularly high that subjects began to opt out of an average of 3 or 4 out of the 10 forced trials. There was a main effect of ratio requirement ($F_{(6, 90)} = 64.72$; $p < 0.001$), indicating that as the number of presses got larger subjects were less willing to complete these trials (Fig 1E). While there was no main effect of hold requirement ($F_{(1, 15)} = 0.002799$; $p = 0.9585$) on opt outs, there was a significant ratio by hold requirement interaction ($F_{(6, 90)} = 7.776$; $p < 0.0001$). There was a slight decrease in the number of opt outs made by subjects when the hold requirement changed from 5 to 10 seconds at the highest ratio requirement, possibly suggesting that changing the perceived effort of one requirement alters that of the alternative, making the high ratio requirement seem less effortful when the alternative was a 10 second hold.

**Value Manipulations Alter Choice Behavior When Choosing Between Two Alternative Rewards**

Because most of the measures studied in CEC are related to effort expenditure, analysis of behavior on the CVC task focused on a choice of the two reward alternatives, pellets and dippers. To examine the relationship of choice behavior between two reward alternatives we parametrically varied ratio requirements for the pellet reward while maintaining an FR5 for a dipper of sucrose solution. We then measured the proportion of dipper choices for the sucrose solution reward as a function of the pellet ratio requirement under two sucrose solution concentrations, 20% and 5% sucrose (Fig 2A). As the FR requirement to earn a pellet increased,

the likelihood that a subject would complete the FR5 to receive a dipper increased. The concentration of sucrose in the solution given as a dipper reward also affected choice behavior such that a greater sucrose concentration increased the proportion of dipper rewards as a function of the ratio requirement. Analysis of the proportion of dipper choices revealed a main effect of sucrose concentration ($F_{(1, 15)} = 41.65$; $P < 0.0001$), ratio requirement ($F_{(3, 45)} = 92.17$ ; $P < 0.0001$), and a significant ratio by concentration interaction ($F_{(3, 45)} = 18.74$; $p < 0.0001$), suggesting that increasing sucrose concentration significantly decreased the number of presses subjects were willing to make for a pellet depending on the work required to earn a pellet. This implicates effort in differentially affecting choice depending on the sucrose concentration, confirmed by a significant difference in the point of subjective equality, or the number of presses for a pellet perceived to be approximately equal to 5 presses for a sucrose dipper when the sucrose concentration was 20% versus 5% ($t_{(15)} = 4.753$; $p = 0.0003$; Fig 2B).

**The Dopamine D2R antagonist Haloperidol affects choice behavior in the Concurrent Effort Choice (CEC) task.**

The proportions of rewards earned by employing a hold lever was impacted by haloperidol, as indicated by a leftward shift in the proportion of hold choices (Fig 3A). There was a main effect of drug on the proportion of rewards earned employing the hold lever ($F_{(2,30)} = 7.710$; $p = 0.0020$), a main effect of ratio requirement ($F_{(3, 45)} = 26.16$; $p < 0.0001$), but no significant drug by ratio interaction ($F_{(6,90)} = 0.2.096$; $p = 0.0614$), suggesting that drug treatment is largely responsible for the shift in choice behavior. Analysis of the point of subjective equality, or the approximated number of responses on the ratio bar equal to the 10-second hold requirement, revealed a significant difference in PSE between treatment with vehicle and each of the two doses of haloperidol (0.1 mg/kg $p < 0.0001$, 0.2 mg/kg $p = 0.0097$; Fig 3B).

Analysis of the number of responses made on the ratio lever across FR requirements revealed differences in trends between groups. While the number of presses made increased as ratio requirement increased when treated with vehicle, subjects treated with 0.2 mg/kg Haloperidol showed a decrease in presses as ratio requirement increased, and pressing behavior exhibited an inverse U-shaped curve across ratio requirements when subjects were treated with 0.1 mg/kg Haloperidol. Statistical analysis revealed a main effect of drug on the number of presses made ($F_{(2,30)} = 18.73$; $p < 0.0001$), no effect of ratio requirement ( $F_{(3, 45)} = 1.119$; $p = 0.3515$), and a drug by ratio interaction ($F_{(6,90)} = 2.237$; $p = 0.0466$; Fig 3C). Subjects experienced differential hold efficiencies with different doses of Haloperidol as well. Hold efficiency was particularly high for subjects when treated with Haloperidol at low ratio requirements, an effect which diminished as these requirements increased. Hold efficiency increased across ratio requirements when subjects were treated with vehicle, reflecting a similar pattern to Fig 1D. Statistical analysis revealed a main effect of drug on hold efficiency ($F_{(2,30)} = 3.497$; $p = 0.0431$), a main effect of ratio requirement ( $F_{(3, 45)} = 4.950$; $p = 0.0047$), and a drug by ratio interaction ($F_{(6,90)} = 5.033$; $p = 0.0002$; Fig 3D).

The number of opt outs taken by each group out of the ten forced trials revealed a dose response effect of the drug. There was a main effect of drug ($F_{(2,30)} = 82.44$; $p < 0.0001$), ratio requirement ( $F_{(3, 45)} = 28.60$; $p < 0.0001$), and a significant drug by ratio interaction ($F_{(6,90)} = 15.20$; $p < 0.0001$) on opt-outs taken (Fig 3E). While differences between vehicle and 0.1 mg/kg Haloperidol treatments on the number of opt outs taken only appeared at high ratio requirements, the 0.2 mg/kg Haloperidol treatment appeared to increase the number of opt outs taken across all ratio requirements. This is further confirmed by analysis of the number of choice trials completed by subjects across ratio requirements in each drug treatment. While subjects appeared

to be able to complete nearly all choice trials in a session when treated with vehicle and 0.1 mg/kg Haloperidol, the number of choice trials completed by subjects treated with 0.2 mg/kg Haloperidol decreased substantially as ratio requirement increased. There was a significant main effect of drug on the number of choice trials the subjects completed ($F_{(2,30)} = 94.70$; $p < 0.0001$), as well as a main effect of ratio requirement ($F_{(3, 45)} = 10.08$; $p < 0.0001$), and a significant drug by ratio interaction ($F_{(6,90)} = 12.11$; $p < 0.0001$; Fig 3F). These results taken in tandem suggest that while the lower dose of Haloperidol may alter choice behavior but not general activity, the higher dose of Haloperidol may actually affect subjects to the point where they stop behaving. Because choice behavior was analyzed using only the trials completed by subjects, the overall findings of altered choice are not impacted by a general decrease in behaving. However, because of this finding, subjects tested on the value choice task later were not tested on the higher dose of Haloperidol.

**The Dopamine D2R antagonist Haloperidol alters choice behavior in the Concurrent Value Choice (CVC) task.**

Similarly to trends observed in the proportion of dipper choices across pellet ratio requirements in Fig 2, the proportion of dipper choices increased as both concentration of sucrose in the dipper reward and pellet cost increased. Under both sucrose concentrations, treatment with Haloperidol tended to shift the proportion of dipper choices left such that subjects were switching over to the dipper reward at lower press requirements. This shift in each vehicle curve with treatment of 0.1 mg/kg Haloperidol reflects a shift in choice behavior due to altered assessment of the effort required to get a pellet, overcoming the value derived from the preferred pellet reward. Raising the sucrose concentration for the dipper reward to 20% increased the proportion of dipper rewards for both vehicle and Haloperidol treatments. A three-way ANOVA

analyzing the effects of pellet ratio requirement, the concentration of the sucrose in the dipper reward, and the drug treatment revealed significant main effects of all three ($F_{(3, 3)} = 129.6$; $p < 0.0001$; $F_{(1, 3)} = 200.2$; $p < 0.0001$; $F_{(1, 3)} = 29.31$; $p < 0.0001$, respectively; Fig 4A). There was a significant pellet ratio requirement by sucrose concentration interaction ($F_{(3, 3)} = 1.52$; $p = 0.0012$), but no significant pellet ratio requirement by drug treatment interaction, sucrose concentration by drug treatment interaction, or pellet ratio by sucrose concentration by drug treatment interaction, suggesting that the effort-related alterations caused by acute antagonism of the D2R are responsible for the leftward shift in choice behavior on the CVC task.

These results are reflected in the point of subjective equality between treatments with vehicle and 0.1 mg/kg Haloperidol across 5% and 20% sucrose concentrations. There was no significant main effect of drug ($F_{(1,13)} = 0.7606$; $p = 0.3989$), a significant main effect of sucrose concentration ($F_{(1,13)} = 6.449$; $p = 0.0247$), and no significant drug by sucrose concentration interaction ($F_{(1,13)} = 0.00555$; $p = 0.9417$; Fig 4B). Post-hoc analysis revealed no statistically significant difference between PSEs across drug treatments or sucrose concentration, making the relationship between Haloperidol and the CVC task less clear.

**Striatal Dopamine D2R overexpression affects choice behavior in the Concurrent Effort Choice (CEC) task.**

While data was collected for ratio requirements across FH05, FH10, and FH20 second requirements, the data presented in the text focuses on the FH20 second data set, as it is representative of all hold requirements in most measurements. Results of the effects of striatal D2R overexpression on the CEC task when the hold alternative was FH05 and FH10 can be found in Appendix A. Similar to the trend in Fig 1A, the proportion of rewards earned using the hold requirement bar increased as ratio requirement increased. However, mice with striatal D2R

overexpression (D2R-OEs) employ hold levers at higher proportions across ratio requirements as compared to other groups. There was a significant main effect of genotype ($F_{(3, 26)} = 21.70$; $p < 0.0001$), ratio requirement ($F_{(6,156)} = 79.00$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(18,156)} = 4.586$; $p < 0.0001$; Fig 5A). Comparing the PSE of each genotype across hold requirements (FH05, FH10, and FH20) revealed that the D2R-OEs equivocate each hold requirement with a decreased press requirement as compared to the other groups for that hold requirement. There was a significant main effect of genotype ($F_{(3, 24)} = 11.05$; $p < 0.0001$), fixed hold requirement ($F_{(2, 48)} = 33.76$; $p < 0.0001$), and a significant genotype by hold interaction ($F_{(6, 48)} = 3.972$; $p = 0.0026$; Fig 5B).

We then separated out subjects by groups and analyzed the proportion of hold choices made per group across the hold requirements tested: FH05, FH10, and FH20. Both control groups, controls fed chow and those fed DOX, employed the hold bar to complete choice trials at the highest frequencies when the hold requirement was lowest at most, but particularly at high, ratio requirements. In the control chow group, there was a significant main effect of hold requirement ($F_{(2, 10)} = 57.07$; $p < 0.0001$), ratio requirement ($F_{(4, 20)} = 45.68$; $p < 0.0001$), and a significant ratio by hold interaction ($F_{(8, 40)} = 6.368$; $p < 0.0001$; Fig 5C). Similarly, the control chow group was significantly influence in their choice behavior by hold requirement ($F_{(2, 14)} = 30.25$; $p < 0.0001$), ratio requirement ($F_{(4, 28)} = 99.61$; $p < 0.0001$), and the interaction of the two ($F_{(8, 56)} = 13.86$; $p < 0.0001$; Fig 5D). Importantly, while this trend followed for D2R-OE subjects on DOX, where there was a significant main effect of hold requirement ($F_{(2, 14)} = 15.95$; $p = 0.0002$), a main effect of ratio requirement ($F_{(4, 28)} = 45.2$; $p < 0.0001$) and a significant hold by ratio interaction ($F_{(8, 56)} = 2.849$; $p = 0.0100$; Fig 5F), there was no significant main effect of hold requirement on the proportion of rewards earned employing the hold bar for D2R-OE subjects on

chow ($F_{(2, 14)} = 1.729$; $p = 0.2133$). There remained a main effect of ratio requirement ($F_{(4, 28)} = 46.91$; $p < 0.0001$) and a significant interaction ($F_{(8, 56)} = 3.698$; $p = 0.0016$; Fig 5E). This is important to note as while these control groups exhibit a characteristic concave curve as ratio requirements increase from relative insensitivity at FR05 and FR10, D2R-OE chow subjects' choice behavior creates a convex curve that asymptotes at maximal hold choice behavior at high ratio requirements, suggesting a left shift in curves of choice across hold requirements not present in control groups. Most interestingly, this left shift in curve shape appears to an intermediate degree in the choice behavior of D2R-OEs on DOX across hold requirements, further evidenced by intermediate points of subjective equality for the D2R-OE DOX group in PSE across hold requirements. This intermediate shift in behavior could indicate some aspects of effort calculation altered by developmental striatal D2R overexpression are irreversible with the DOX regimen.

The trend of D2R-OE subjects fed chow to elect to earn reinforcers using the hold bar more frequently at lower press requirements is reflected in analysis of the number of responses made on the press lever. The number of presses across ratio requirement revealed that while controls and D2R-OEs fed DOX increased pressing until FR80, at which point the number of responding on the ratio bar decreased as subjects began employing the FH requirement, D2R-OEs on chow had limited responding on the ratio bar across all FR requirements. There was a main effect of Genotype ($F_{(3, 26)} = 7.523$; $p = 0.0009$), Ratio Requirement ($F_{(6,156)} = 17.67$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(18,156)} = 2.791$; $p = 0.0003$; Fig 6A). This evidence further confirms previous research that D2R-OE mice perform poorly on ratio tasks, a deficit rescued by reversing the overexpression (Drew et al., 2007).

In contrast, D2R-OEs exhibited robustly increased hold efficiency as compared to other groups across hold requirements. Similarly to Fig 1D, hold efficiency for controls and D2R-OEs on DOX increased across ratio requirements; this trend did not apply to the D2R-OEs on chow. There was a significant main effect of genotype on hold efficiency ($F_{(3, 26)} = 15.47$; $p < 0.0001$), a significant main effect of ratio requirement ($F_{(5, 130)} = 26.85$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(15, 130)} = 3.423$; $p < 0.0001$; Fig 6B), further confirming previous research suggesting that the D2R-OE mice perform as well, if not better than control mice, on tasks that reinforce bar holding (Unpublished Data).

Like our preliminary data had shown, the number of opt outs taken out of the 10 forced trials did not vary much across ratio requirements for control mice and D2R-OE mice on DOX. However, as ratio requirements increased, particularly at FR40 and beyond, the D2R-OE mice fed chow appeared to increase the number of opt outs they took. There was no main effect of genotype ($F_{(3, 26)} = 1.905$; $p = 0.1535$), but a main effect of ratio requirement ($F_{(6,156)} = 39.08$; $p < 0.0001$), and a significant genotype by ratio requirement interaction ($F_{(18,156)} = 4.686$; $p < 0.0001$; Fig 6C). Additionally, examining the number of choice trials showed that subjects again completed fewer choice trials as ratio requirements became more demanding. D2R-OEs, however, appeared to complete fewer choice trials across all ratio requirements. There was a main effect of genotype ($F_{(3, 26)} = 4.388$; $p = 0.0126$), ratio requirement ($F_{(6,156)} = 3.280$; $p = 0.0046$), and a genotype by ratio requirement interaction ($F_{(18,156)} = 1.830$; $p = 0.0260$; Fig 6D).

Further evidence points towards remaining irreversible effects in effort calculation of the D2R-OE DOX group suggested by the intermediate choice behavior of the D2R-OE DOX group compared to controls and D2R-OEs on chow. No significant differences were found in the number of presses made between the D2R-OE chow and D2R-OE DOX groups, and at FR80,

there was a significant difference between Control DOX and D2R-OE DOX groups (Fig 6A). Similarly, as ratio requirement increases, the hold efficiency of D2R-OE subjects on DOX increases to be not significantly different than D2R-OEs on chow at FR40, while the control DOX group especially maintain their difference in hold efficiency (Fig 6B).

**Striatal Dopamine D2R overexpression does not alter choice in the Concurrent Value Choice (CVC) task.**

To examine whether striatal D2R overexpression impacts the calculation of value, we measured the proportion of dipper choices made by the different groups. As a general trend, similarly to preliminary studies of the CVC task, as the pellet requirement increased, the proportion of dipper choices increased across all groups. When the concentration of the sucrose solution was 20%, there was no main effect of genotype ($F_{(3, 24)} = 1.128$; $p = 0.3575$), but a main effect of ratio requirement ($F_{(4, 96)} = 42.09$; $p < 0.0001$), and no significant genotype by ratio interaction ($F_{(12, 96)} = 0.8630$; $p = 0.5865$; Fig 7A), suggesting that the D2R-OE mice were not choosing differently as compared to controls and D2R-OE mice fed DOX. Comparing choice behavior between genotypes across sucrose concentrations through the PSE revealed a decrease in the extrapolated PSE, which reflects the hypothetical presses for a pellet a subject considers equal to a sucrose dipper, from controls fed chow as compared to the other groups in both concentrations of sucrose. There was a main effect of Genotype ($F_{(3, 22)} = 4.439$; $p = 0.0139$), a main effect of sucrose concentration ($F_{(1, 22)} = 32.60$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(3, 22)} = 4.572$; $p = 0.0123$; Figure 7B), such that controls fed chow had a significantly higher PSE as compared to the other groups when the dipper concentration was 5%, suggesting that controls on chow were particularly unwilling to switch over to earn their rewards as dippers.

Further analysis of this difference was made possible by separating the subject groups and comparing choice behavior in the CVC task across the sucrose concentrations given in the dipper reward. In all comparisons, subjects chose to earn rewards as dippers at increased frequencies when the concentration of the sucrose dipper reward was 20% as compared to 5% at low pellet ratio requirements. As pellet ratio requirements increased, each subject group appeared to behave differently. The controls on chow, for example, maintained relatively parallel patterns of choice between sucrose concentrations, with a significant difference between proportion of dipper choices even when the work requirement for pellets was high: within this group, there was a main effect of pellet ratio requirement ($F_{(4, 24)} = 14.82$; $p < 0.0001$), a main effect of sucrose concentration ($F_{(1, 6)} = 32.99$; $p = 0.0012$), and no significant interaction ($F_{(4, 24)} = 1.564$; $p = 0.2160$; Fig 7C). This differs from the choice behavior found in the D2R-OE chow group, where at high pellet ratio requirements, there was no significant difference in choice behavior between sucrose concentrations; however within this group, two-way ANOVA analysis revealed similar trends; there was a main effect of pellet ratio requirement ($F_{(4, 20)} = 28.39$; $p < 0.0001$), a main effect of sucrose concentration ($F_{(1, 5)} = 135.3$; $p < 0.0001$), and no significant interaction ($F_{(4, 20)} = 0.2141$; Fig 7E). Interesting, both groups treated with DOX exhibited altered choice behavior based on the pellet ratio requirement depending on the sucrose concentration in the dipper: in the control group given DOX, there was a main effect of pellet ratio requirement ($F_{(4, 32)} = 49.84$; $p < 0.0001$), a main effect of sucrose concentration ($F_{(1, 8)} = 83.42$; $p < 0.0001$), and a significant interaction ($F_{(4, 32)} = 5.187$; $p = 0.0025$; Fig 7D), while the choice behavior of D2R-OE subjects fed DOX was significantly affected by pellet ratio requirement ($F_{(4, 20)} = 28.2$; $p < 0.0001$), sucrose concentration ($F_{(1, 5)} = 25.81$; $p = 0.0038$), and a significant interaction ($F_{(4, 20)} = 8.283$; $p = 0.0004$; Fig 7F). These differential outcomes based on group suggests

correspondingly different sensitivities to change in sucrose concentration based on the effort required to complete the work requirement for the preferred pellet reward. This is particularly evident at pellet ratio requirement FR40, where both D2R-OE groups no longer choose differently from one sucrose concentration to the other. This similarity in both D2R-OE groups' choice behavior may suggest a similarly altered sensitivity to work requirement when work reaches a threshold effort.

## Figure 1

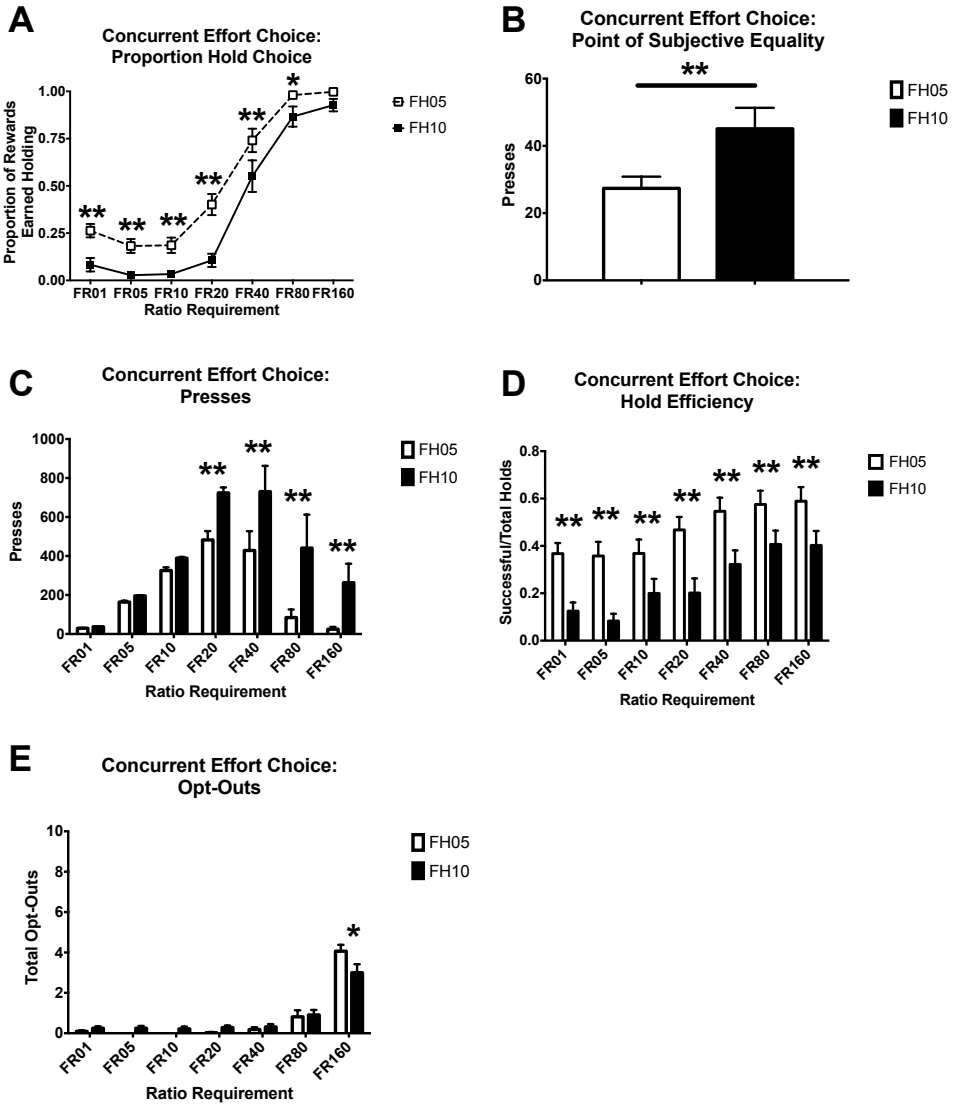


**Figure 1. Choice behavior of subjects offered concurrent FR and FH work alternatives on the CEC task. A.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with a five (FH05; dashed) and ten (FH10, solid) fixed hold requirement. **B.** Mean (±SEM) point of subjective equality, the number of presses approximated to yield a 50% chance of using FR or FH schedules for FH05 (white) and FH10 (black). **C.** Mean (±SEM) responses made on the ratio bar for fixed ratio schedules offered concurrently with FH05 (white) and FH10 (black). **D.** Mean (±SEM) successful/total responses made on the hold bar for fixed ratio requirements offered concurrently with FH05 (white) and FH10 (black). **E.** Mean (±SEM) number out of 10 total forced trials subjects subjects did not complete within 3 minutes (designated an "opt out") for fixed ratio schedules offered concurrently with FH05 (white) and FH10 (black). Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

## Figure 2

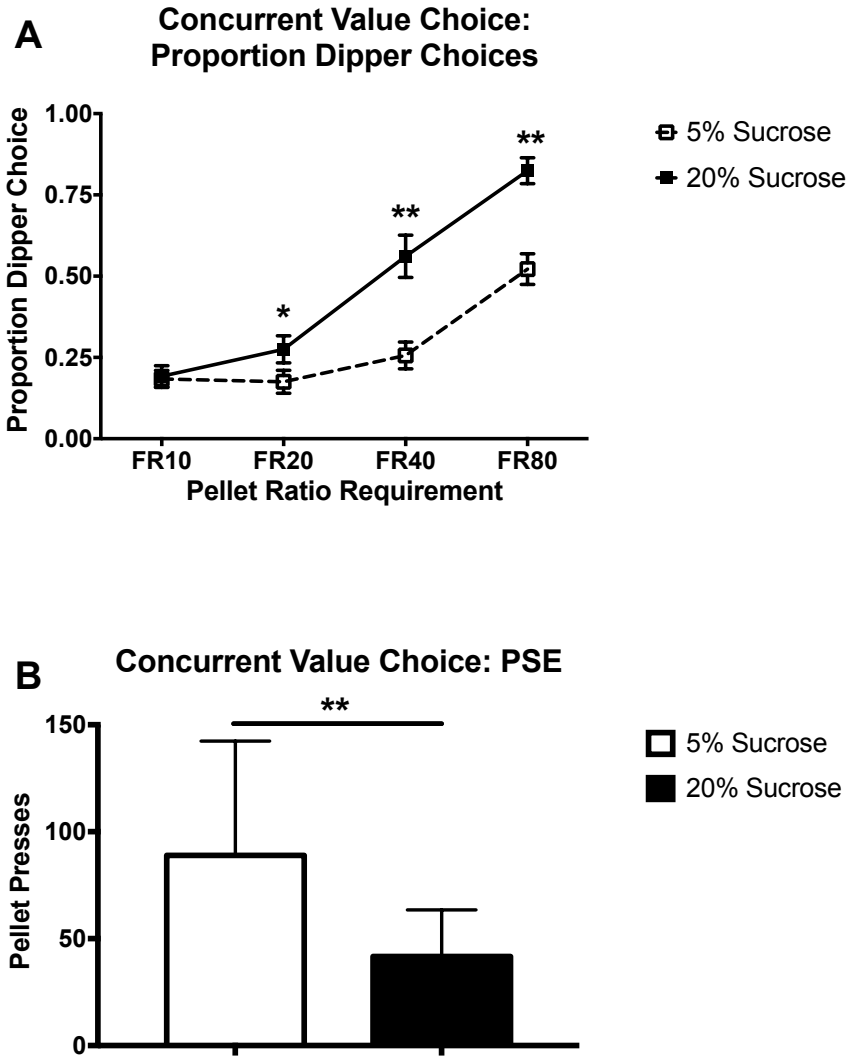


**Fig 2. Choice behavior of subjects offered concurrent FR schedules for pellet and dipper reward alternatives on the CVC task. A.** Mean (±SEM) proportion of rewards earned as a dipper on an FR5 for fixed ratio schedules for a pellet reward when the concentration of sucrose in the dipper reward was 5% (empty) and 20% (filled). **B.** Mean (±SEM) point of subjective equality, the number of presses for a pellet a subject would calculate to be equal to an FR5 for 5% (white) and 20% (black) sucrose dipper. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

# Figure 3

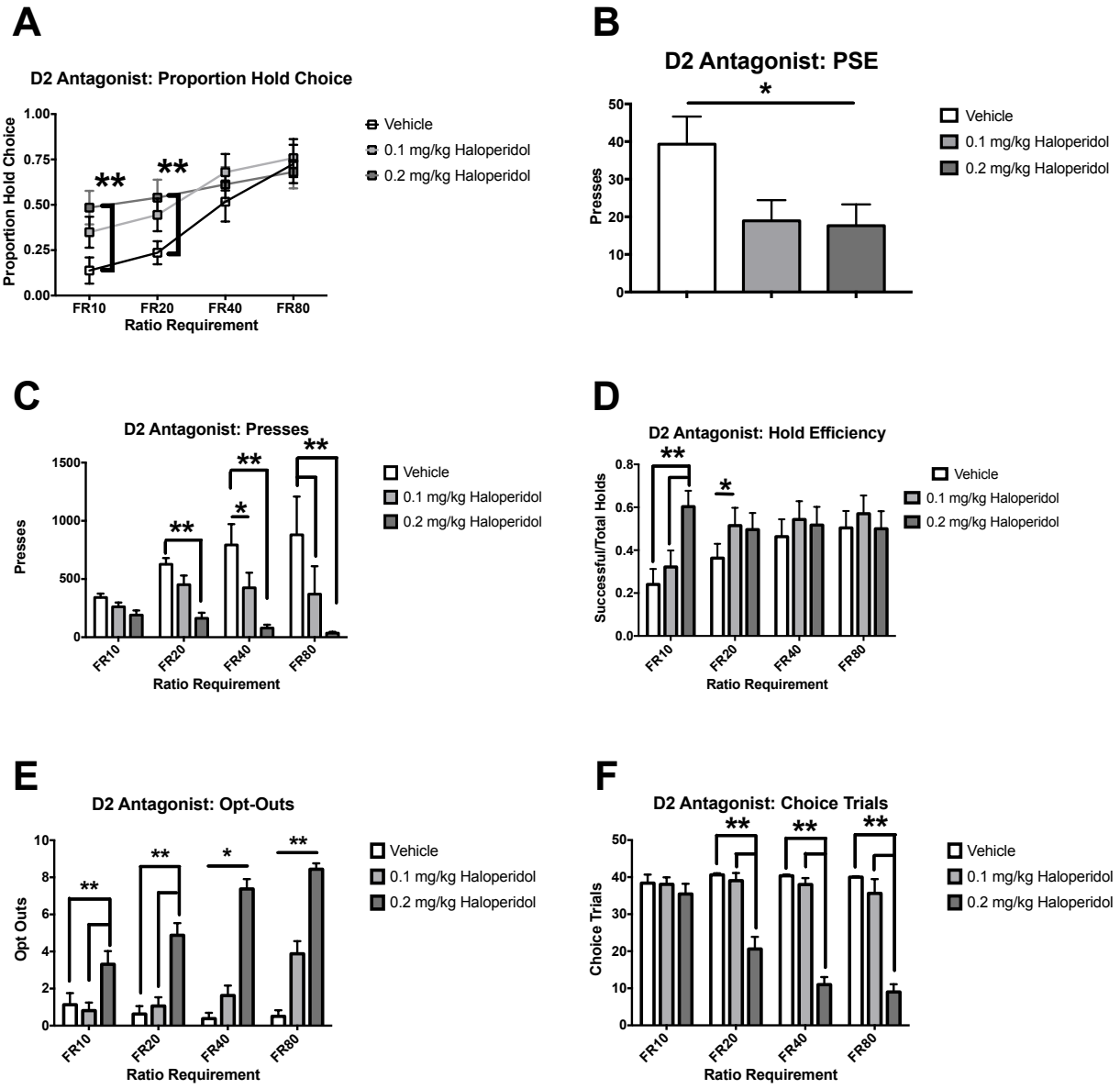


**Figure 3. The effects of the D2R antagonist Haloperidol on behavior in the CEC task. A.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with FH10 for subjects treated with vehicle (empty), 0.1 mg/kg (light gray), and 0.2 mg/kg (dark gray) D2R antagonist Haloperidol. **B.** Mean (±SEM) point of subjective equality on FH10. **C.** Mean (±SEM) responses made on the ratio bar for fixed ratio schedules offered concurrently with FH10. **D.** Mean (±SEM) successful/total responses made on the hold bar for fixed ratio requirements offered concurrently with FH10. **E.** Mean (±SEM) opt outs for fixed ratio schedules offered concurrently with FH10. **F.** Mean (±SEM) choice trials completed for fixed ratio schedules offered concurrently with FH10. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

## Figure 4

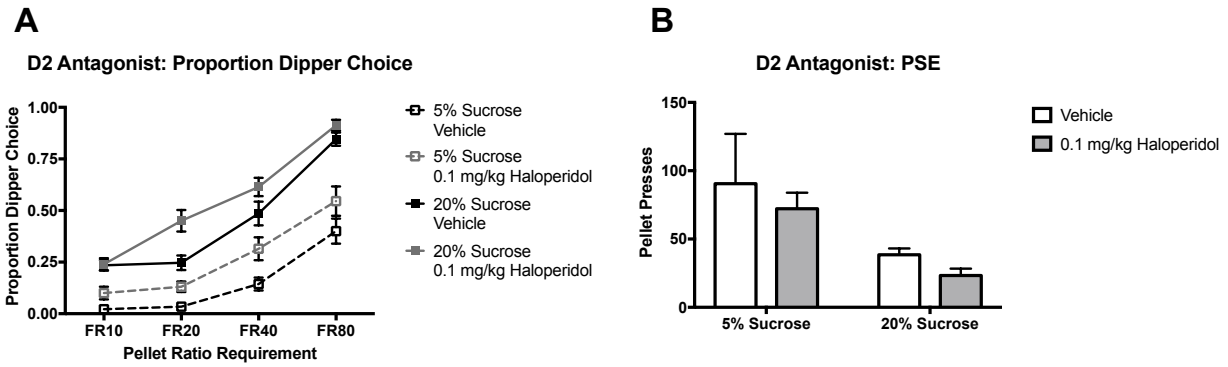


**Figure 4. The effects of the D2R antagonist Haloperidol on choice behavior in the CVC task. A.** Mean (±SEM) proportion of rewards earned as a dipper on an FR5 for fixed ratio schedules for a pellet reward when the concentration of sucrose in the dipper reward was 5% and 20% sucrose for subjects treated with vehicle (empty) or 0.1 mg/kg (light gray) D2R antagonist Haloperidol. **B.** Mean (±SEM) point of subjective equality for subjects treated with vehicle and 0.1 mg/kg Haloperidol for 5% and 20% sucrose solutions. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

# Figure 5

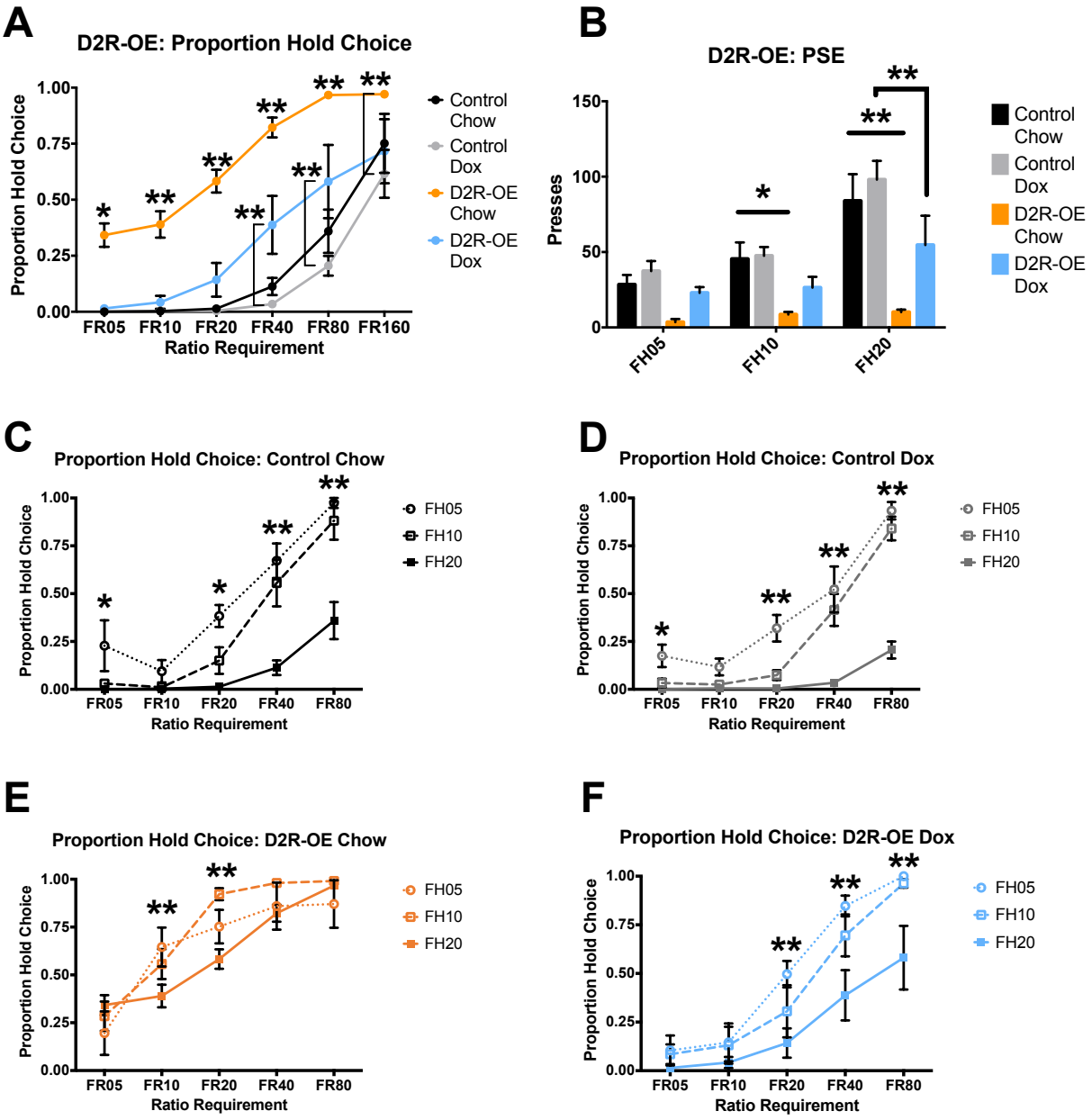


**Figure 5. The effects of striatal D2R overexpression on choice behavior in the CEC task. A.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with FH20 for controls on chow (black), controls on doxycycline (DOX; gray), D2R-OEs on chow (orange), and D2R-OEs on DOX (blue). **B.** Mean (±SEM) point of subjective equality on FH20 **C.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with FH20 for controls on chow; **D.** controls on DOX; **E.** D2R-OEs on chow; **F.** D2R-OEs on DOX. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

# Figure 6

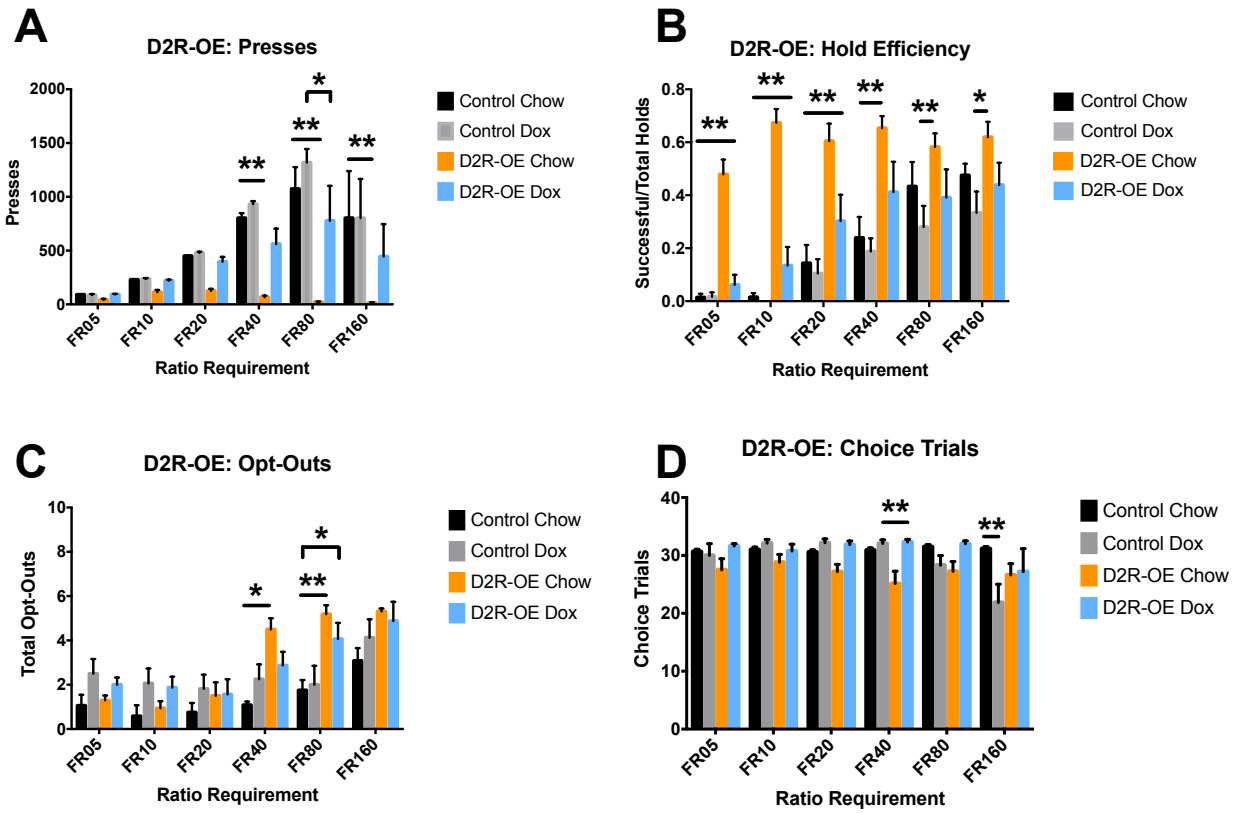


**Figure 6. The effects of striatal D2R overexpression on behavior in the CEC task. A.** Mean (±SEM) responses made on the ratio bar for fixed ratio schedules offered concurrently with FH20. **B.** Mean (±SEM) successful/total responses made on the hold bar for fixed ratio requirements offered concurrently with FH20. **C.** Mean (±SEM) opt outs for fixed ratio schedules offered concurrently with FH20. **D.** Mean (±SEM) choice trials completed for fixed ratio schedules offered concurrently with FH20. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

# Figure 7

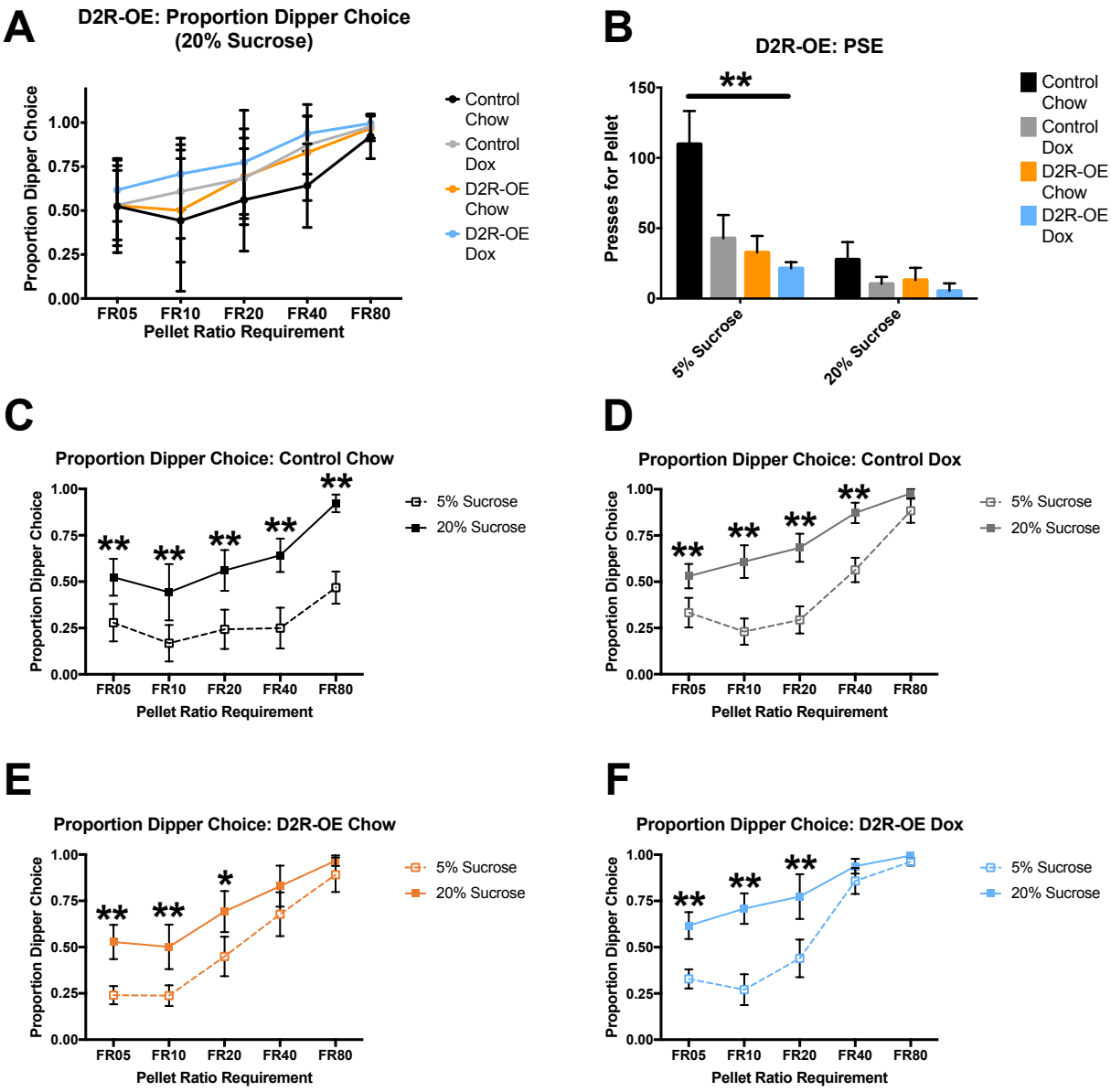


**Figure 6. The effects of striatal D2R overexpression on choice behavior in the CVC task. A.** Mean (±SEM) proportion of rewards earned as a dipper on an FR5 for fixed ratio schedules for a pellet reward when the concentration of sucrose in the dipper reward was 20% sucrose for controls on chow (black), controls on doxycycline (DOX; gray), D2R-OEs on chow (orange), and D2R-OEs on DOX (blue). **B.** Mean (±SEM) point of subjective equality across groups for 5% and 20% sucrose solutions. **C.** Mean (±SEM) proportion of rewards earned as a dipper on an FR5 for fixed ratio schedules for a pellet reward when the concentration of sucrose in the dipper reward was 5% or 20% sucrose for controls on chow; **D.** controls on DOX; **E.** D2R-OEs on chow; **F.** D2R-OEs on DOX. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

## *Discussion*

### Methods for studying effort- and value-based choices

The Concurrent Choice tasks give animals a choice between different types of alternatives: while the Concurrent Effort Choice (CEC) task gives subjects a choice between two different response types, the Concurrent Value Choice (CVC) task offers a choice between two reward alternatives. Thus, these two tasks dissociate the distinct processes of effort- and value-based decision making in motivated behavior.

The CEC task turns out to be a sensitive assay of effort. Subjects earned a greater proportion of rewards with the hold lever as the ratio requirement increased. When the effort requirement was increased by increasing the duration of the hold required to get a reward, the psychophysical function describing the choice to earn reinforces by producing a successful hold shifted to the right, indicating that subjects were willing to make more press responses as the hold requirement increased. This is concisely represented in the rightward shift of the point of subjective equality (PSE), representing that increasing hold requirement from FH05 to FH10 subsequently increased the PSE from 27 to 45 presses.

Similarly, the CVC task was sensitive to changes in value. This was indicated by a leftward shift in the psychophysical function representing sucrose choice versus pellets produced by increasing the value of the dipper alternative by increasing the concentration of sucrose reward. This is reflected in a decrease in the PSE, showing that increasing sucrose concentration from 5% to 20% decreased the average PSE from 89 to 42 presses.

Importantly, there is an apparent change in the slope of the functions representing the proportion of rewards earned as sucrose dippers. When the cost of a pellet is low, subjects mainly choose to press for pellet rewards for both sucrose values. However, as it becomes

effortful to earn a pellet, the proportion of rewards earned as a dipper when the concentration of sucrose in the dipper is 5% is significantly different than 20% sucrose, suggesting that differences in reward value may only be reflected in choice when there is a significant difference in effort to obtain the preferred reward. These findings contributed to our understanding of the role of effort and value in choice which had been previously unknown due to the coupling of effort and value in behavioral paradigms (Gold et al., 2015).

**The role of acute D2R antagonism in effort and value calculation**

Acute antagonism of the D2R was found to produce a leftward shift in the psychophysical function of hold choice behavior on the CEC task reflected in the decrease in PSE with treatment of both 0.1 and 0.2 mg/kg Haloperidol from 39 presses at baseline to 19 and 18 presses, respectively. It was found through analysis of general behavior in the CEC task that the 0.2 mg/kg Haloperidol dose produced a general disruption of behavior. This was evidenced by a decreased number of presses, substantially increased number of opt-outs, and a decreasing number of choice trials completed per session across ratio requirements. However, treatment with 0.2 mg/kg Haloperidol did not reduce hold efficiency across ratio requirements, suggesting that the shift in choice was not a result of a general disruption of behavior. Furthermore, choice behavior across tasks and manipulations were measured by proportions of the total number of completed trials, so this general behavior did not enter into the analysis of choice behavior. In sum, these findings suggest that the assessment of the effort required to produce repetitive initiation of responding is affected by acute D2R antagonism, altering the relationship between bar holding and bar pressing.

Similarly to the baseline findings of the CVC task (Fig 2), there was a differential effect of ratio requirement on choice behavior depending on the concentration of sucrose in the dipper reward. This was reflected in a significant sucrose concentration by pellet ratio requirement interaction. Acute D2R antagonism with Haloperidol produced a leftward shift in the psychophysical functions representing dipper choice under both sucrose concentrations such that treatment with Haloperidol was making working for pellets appear more costly. This in tandem with the shift in choice produced by Haloperidol on the CEC task suggests acute D2R antagonism alters choice behavior on the CVC task by altering the assessment of effort required to receive a pellet, not by changing reward values

That acute D2R antagonism alters behavior away from high work requirements is in agreement with previous research that dopamine antagonists and depletion decreases willingness to work on the effort-based choice (EBC) task for the preferred reward Salamone et al., 1991, 2002; Koch et al. 2000; Nowend et al. 2001; Sink et al. 2008; Farrar et al. 2010). Furthermore, D2R antagonism specifically decreases the likelihood of choosing the high-effort/high-reward arm in the T-maze barrier task (Bardgett et al., 2009; Salamone et al. 1994; Cousins et al. 1996; Mott et al. 2009; Mai et al. 2012; Pardo et al., 2012), decreases lever pressing in EBC (Salamone et al., 1991) and decreases responding in progressive ratio (PR) tasks (Aberman et al., 1998), consistent with the shift in the psychophysical function of hold choice behavior.

**The effect of chronic striatal D2R overexpression and the calculation of effort and value**

Similarly to pharmacological D2R antagonism, striatal D2R overexpression was found to produce a leftward shift in the psychophysical function of proportion of hold choices, reflected in a lower PSE that averaged 10 presses for D2R-OE subjects provided chow as compared to an

average of 84 presses for controls on chow. The sensitivity to hold requirements was evident in all groups, as shown by rightward shifts in the function of proportion of rewards earned holding across ratio requirements as the alternative hold requirement increased in duration. The D2OE subjects showed the least sensitivity to the increased hold requirements and preferred holding as the option to earn rewards more than the other groups—even at the highest hold requirement tested. This difference in behavior is reflected in various measures of effort expenditure, D2R-OEs on chow made substantially fewer responses on the ratio lever at high ratio requirements and yet exhibit consistently high hold efficiency at low ones, while controls and exhibit a characteristic increase in responding across ratio requirements, and subsequent increase hold efficiency as bar holding becomes their primary mode of work. In particular, D2R-OE subjects on chow were consistently efficient in making successful holds across ratio requirements and more efficient than controls and D2R-OEs on DOX at low ratio requirements, suggesting that altered choice is not the result of a general motor deficit but rather due to altered effort assessment of the relationship between types of work.

D2R-OE subjects given a doxycycline (DOX) were sensitive to the differences between FH05 and FH20 only at higher ratio requirements. This is reflected in both significant differences in the proportion of rewards earned holding between D2R-OEs on DOX and controls at intermediary ratio requirements and a PSE of the D2R-OE DOX group that, while not significantly different than the control groups, was intermediate between the D2R-OE chow and control groups. At FH05 the PSE for control chow and DOX groups are 28 and 37, respectively, the D2R-OE DOX group does not differ significantly at 23 presses (compared to 4 presses for D2R-OEs on chow). However, this is in contrast to PSEs at FH20: 84 and 98 for controls on chow and DOX, 10 for D2R-OEs on chow, and 55 for D2R-OEs on DOX. These findings

suggest a potentially irreversible effect of developmental striatal D2R overexpression on the assessment of effort. This is further supported by the fact that the D2R-OE DOX group exhibits intermediary pressing behavior at higher ratio requirements, actually being significantly different than the control DOX group at FR80.

Similarly to the baseline findings of the CVC task (Fig 2), there was a differential effect of ratio requirement on choice behavior depending on the concentration of sucrose in the dipper reward. This was reflected in a significant sucrose concentration by pellet ratio requirement interaction. Acute D2R antagonism with Haloperidol produced a leftward shift in the psychophysical functions representing dipper choice under both sucrose concentrations such that treatment with Haloperidol was making working for pellets appear more costly. This in tandem with the shift in choice produced by Haloperidol on the CEC task suggests acute D2R antagonism alters choice behavior on the CVC task by altering the assessment of effort required to receive a pellet.

Chronic striatal D2R overexpression appeared to influence choice behavior on the CVC task, as increasing work requirements to get a pellet decreased the difference in choice behavior between the two concentrations of sucrose provided in the dipper reward. Particularly at high pellet costs (FR40 and FR80), there was no significant difference in the proportion of dipper choices between sucrose concentrations for the D2R-OE chow subjects. This contrasts to significant differences in choice behavior across ratio requirements between the two sucrose concentrations in the control chow group, a pattern also exhibited by the control DOX group except at the highest ratio requirement where they were more like the D2R-OE chow subjects in showing not difference between sucrose concentrations. This suggests that not only chronic D2R overexpression affects the effort assessment involved in making the work towards a valued

choice but also again suggests an irreversible effect of developmental D2R overexpression in effort assessment.

That D2R-OEs on DOX may show irreversible, residual effects from developmental striatal D2R overexpression is in opposition to previous studies of the D2R-OE mouse model in operant behavior. Previous research studying motivation in the D2R-OE model has shown that the motivational deficits observed in D2R-OE were rescued with treatment of DOX in both the progressive ratio (PR) task (Drew et al., 2007, Simpson et al, 2011) and the effort-based choice (EBC) tasks (Ward et al., 2012). However, Drew et al. (2007) found that D2R-OE subjects on DOX did make fewer lever presses than control subjects but more than D2R-OEs on chow on the progressive ratio task, suggesting a similar finding of intermediary performance on behavioral tasks measuring motivated behavior (Drew et al, 2007). It is possible that the D2R-OEs have residual sensitivity to high work requirements, particularly when required to repeatedly initiate responding, as a result of irreversible effects from developmental D2R overexpression.

**Considering parallel shifts in choice behavior from acute D2R antagonism and chronic D2R overexpression**

Both acute D2R antagonism and chronic D2R overexpression yielded leftward shifts in choice behavior in both the CEC and CVC tasks. How could altering D2R signaling in opposite directions produce identical behavioral effects? While D2R antagonists were administered acutely in the present experiment, the D2R was overexpressed in subjects developmentally, which has been suggested to result in compensatory mechanisms (Kellendonk et al., 2006). Furthermore, D2R overexpression is restricted to postsynaptic medium spiny neurons of the striatum, while D2R antagonism in the present experiment affected D2R transmission in other subpopulations of neurons within the striatum as well as throughout the remainder of the brain,

including but not limited to the striatonigral and striatopalladial pathways and striatal cholinergic interneurons (Trifilieff et al, 2013). Future research should consider the distinct role of D2Rs in neuronal subpopulations within the striatum in the assessment of effort and value in order to parse apart the directionality of these assessments.

**Implications and future directions**

The series of concurrent choice tasks detailed here offer the field a pair of operant tasks which examine changes in either effort or value while keeping the other constant. Unlike previous tasks in which effort and value are manipulated but not completely dissociated, the CEC and CVC tasks separate calculation of effort and value and measure them quantitatively and parametrically (Gold et al., 2015).

The CEC and CVC tasks represent the first step towards isolating separate components which influence motivated behavior in order to better elucidate how the calculation of effort and value are integrated to influence action. As we develop tasks which better isolate these distinct processes, we will be able to better understand which neural circuits process underlying the cost-benefit analysis that leads to motivated behavior. Future research should attempt to use the CEC and CVC tasks to apply the generalized matching law to analyzes whether subjects exhibit a bias or sensitivity in their choice behavior on the tasks. This would inform whether manipulating the D2 receptor alters bias, the preference for a particular type of work or reward that cannot be accounted for by reinforcement alone, or sensitivity, the change in behavior with change in reinforcement (Baum 1974; Reed and Kaplan, 2011).

Similar leftward shifts in choice behavior due to both acute D2R antagonism and chronic striatal D2R overexpression represents the myriad of ways dopamine and the D2R regulates

effort- and value-based decision making. By better targeting the sources of effort and value assessment through specific manipulations of the D2R, we may be better able to provide pharmacological interventions to psychiatric disorders affecting motivation.

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*Appendix A*

**The effects of striatal D2R overexpression on the CEC task for FH05 and FH10 requirement alternatives**

Similar to previous analyses of the proportion of rewards earned by employing the hold requirement, the proportion increased as the ratio requirement increased when the hold requirement was both FH05 and FH10. In both cases, similar to when the FH20 alternative was given, the D2R-OEs on chow employed the hold lever more frequently. When the hold requirement was FH05, this was revealed in a substantial increase in this proportion across the mid-range ratio requirements (FR10, FR20, FR4). There was a main effect of genotype ($F_{(3,26)}$ = 4.802; p = 0.0086), ratio requirement ($F_{(5, 130)}$ = 95.52; p < 0.0001), and a significant genotype by ratio interaction ($F_{(15,130)}$ = 3.644; p < 0.0001; Fig 8A). This is reflected in the average PSE for groups when the hold requirement was 5 seconds. There is a significant decrease in the number of presses equal to a 5 second hold for the D2R-OE mice on chow as compared to controls on chow, controls on DOX, and D2R-OEs on DOX (p = 0.0093; 0.0002; 0.0468; Fig 8B). This change became more pronounced when the hold requirement doubled to FH10: there was a significant main effect of genotype ($F_{(3, 26)}$ = 22.21; p < 0.0001), ratio requirement ($F_{(6, 156)}$ = 215.6; p < 0.0001), and a significant genotype by ratio interaction ($F_{(18,156)}$ = 7.024; p < 0.0001; Fig 9A). This is again reflected in a significant decrease in the PSE of the D2R-OE chow group as compared to the control chow and control DOX group (p = 0.0042; 0.0015; Fig 9B).

Similar trends in pressing and holding behavior are observed in the FH05 and FH10 alternatives as observed in the FH20 alternative (Fig 6A). In all cases, subjects in the D2R-OE chow group have a decline in the number of responses made on the ratio lever across FR requirements as these responses increase to a breakpoint for controls and D2R-OEs on DOX. When the hold requirement was FH05, there was a main effect of genotype ($F_{(3,26)}$ = 9.098; p =

0.0003), ratio requirement ($F_{(5, 130)} = 33.95$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(15,130)} = 6.477$; $p < 0.0001$; Fig 8C). Doubling the FH requirement caused each group to make more presses than they had before, shifting their tendency to employ the hold lever right. However, the trend still remained the same; there was a significant main effect of genotype ($F_{(3, 26)} = 9.259$; $p = 0.0002$), ratio requirement ($F_{(6, 156)} = 24.21$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(18,156)} = 4.193$; $p < 0.0001$; Fig 9C).

There were substantial differences in the hold efficiency of subjects between the FH05 alternative and that of the FH10 alternative. While there appears to be a general increase of hold efficiency as FR requirement increases, there is not a robust difference in hold efficiency at this low duration requirement. This may be because the hold requirement is not long enough to distinguish a differential skill towards bar holding. At the FH05 requirement, there was no main effect of genotype ($F_{(3,26)} = 1.463$; $p = 0.2475$), ratio requirement ($F_{(5, 130)} = 26.59$; $p < 0.0001$), and no significant genotype by ratio interaction ($F_{(15,130)} = 1.259$; $p = 0.2373$; Fig 8D). However, when the FH requirement was doubled, there was a significant main effect of genotype ($F_{(3, 26)} = 13.8$; $p < 0.0001$), ratio requirement ($F_{(6, 156)} = 28.75$; $p < 0.0001$), and no significant genotype by ratio interaction ($F_{(18,156)} = 1.504$; $p = 0.0950$; Fig 9D). In this way, the FH10 alternative reflects the trends of hold efficiency across groups observed in the FH20 alternative, suggesting that there may be a critical point at which D2R-OEs become significantly more efficient at bar holding.

The FH05 and FH10 requirement alternatives to bar pressing show similar outcomes to that of the FH20 requirement in completing the task in general, making very few opt outs and completing most choice trials per session. At FH05, there is very little opting out in the forced trials, until the highest ratio requirements, when opt outs increase across groups. At FH05, there

was a main effect of genotype ($F_{(3,26)} = 6.998$; $p = 0.0013$), ratio requirement ($F_{(5, 130)} = 42.60$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(15,130)} = 3.973$; $p < 0.0001$; Fig 8E). However, at FH10, there are more opt outs taken by every group, even at the lowest ratio requirements, than when the alternative was FH05. This could reflect opting out of the 10 second hold requirement trials. This explanation would reflect the differential hold efficiency across groups, where D2R-OEs on chow are far more efficient at making 10-second holds than other groups. At FH10, there was no significant main effect of genotype ($F_{(3, 26)} = 1.905$; $p = 0.1535$), a main effect of ratio requirement ($F_{(6, 156)} = 39.08$; $p < 0.0001$), and a significant genotype by ratio interaction ($F_{(18,156)} = 4.686$; $p < 0.0001$; Fig 9E). The previous findings using the FH20 requirement's analysis of the number of choice trials completed in the session are reflected in both FH05 and FH10 requirements. There was no main effect of genotype at either hold requirement (FH05: $F_{(3,26)} = 0.6849$; $p = 0.5694$; FH10: $F_{(3, 26)} = 0.2405$; $p = 0.8673$). While there was no significant effect of ratio requirement at FH05, ($F_{(5, 130)} = 1.126$; $p = 0.3498$), there was at FH10 ($F_{(6, 156)} = 18.36$; $p < 0.0001$), and both requirements had no significant genotype by ratio interaction (FH05: $F_{(15,130)} = 0.6259$; $p = 0.8494$; Fig 8F; FH10: $F_{(18,156)} = 1.258$; $p = 0.2232$; Fig 9F). Interestingly, this trend across ratio requirements on the FH10 was in the positive direction such that subjects were completing more choice trials at high ratio requirements. This trend is actually a result of the task design; if a subject made more than five opt outs in a trial, they were given additional choice trials to complete.

# Figure 8

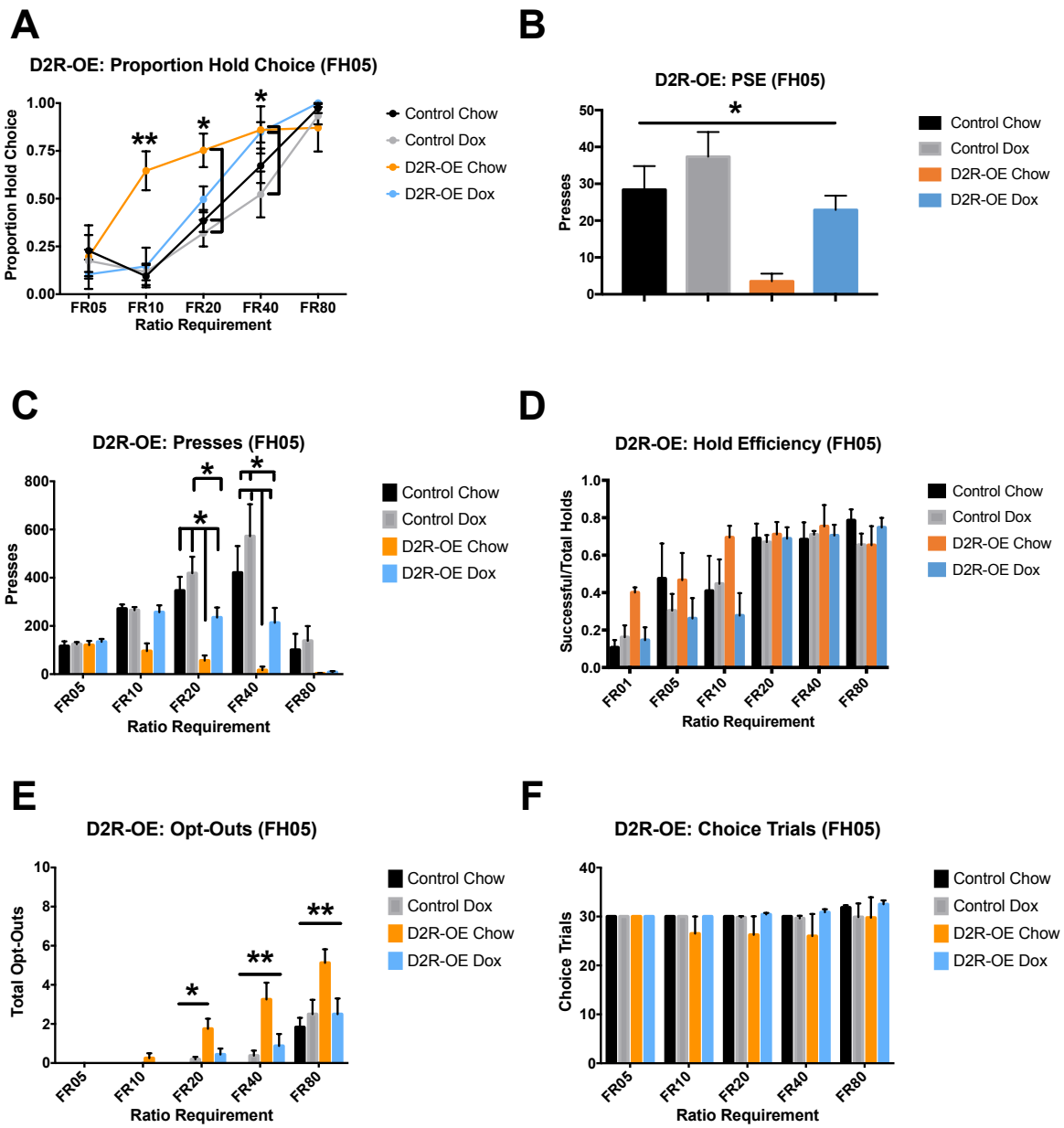


**Figure 8. The effect of striatal D2R overexpression on behavior in the CEC task with a FH05 requirement alternative. A.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with FH05 for controls on chow (black), controls on doxycycline (DOX; gray), D2R-OEs on chow (orange), and D2R-OEs on DOX (blue). **B.** Mean (±SEM) point of subjective equality on FH05 **C.** Mean (±SEM) responses made on the ratio bar for fixed ratio schedules offered concurrently with FH05. **D.** Mean (±SEM) successful/total responses made on the hold bar for fixed ratio requirements offered concurrently with FH05. **E.** Mean (±SEM) opt outs for fixed ratio schedules offered concurrently with FH05. **F.** Mean (±SEM) choice trials completed for fixed ratio schedules offered concurrently with FH05. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

# Figure 9

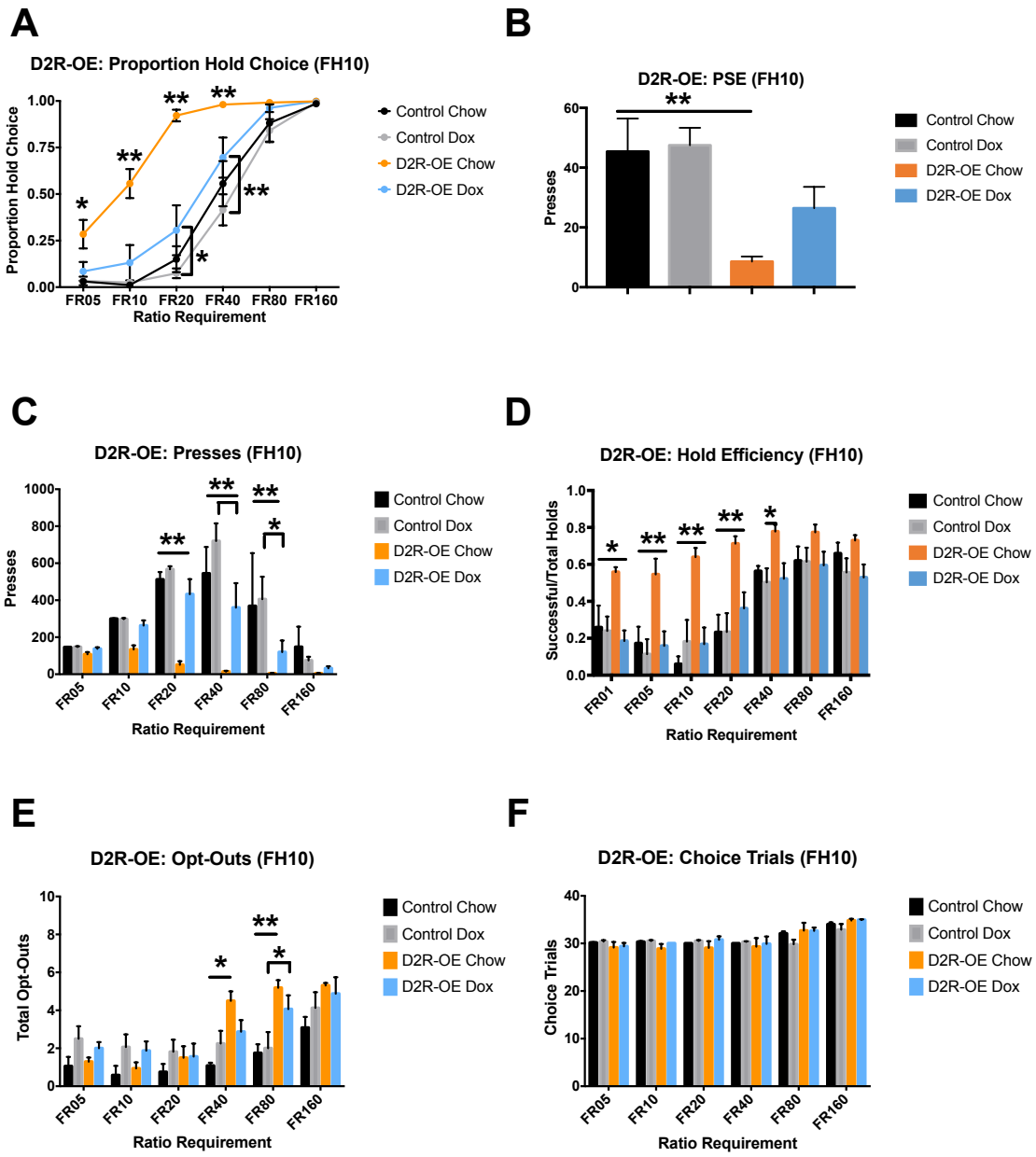


**Figure 9. The effect of striatal D2R overexpression on behavior in the CEC task with a FH10 requirement alternative. A.** Mean (±SEM) proportion of rewards earned by employing the hold bar for each fixed ratio schedule offered concurrently with FH10 for controls on chow (black), controls on doxycycline (DOX; gray), D2R-OEs on chow (orange), and D2R-OEs on DOX (blue). **B.** Mean (±SEM) point of subjective equality on FH10 **C.** Mean (±SEM) responses made on the ratio bar for fixed ratio schedules offered concurrently with FH05. **D.** Mean (±SEM) successful/total responses made on the hold bar for fixed ratio requirements offered concurrently with FH05. **E.** Mean (±SEM) opt outs for fixed ratio schedules offered concurrently with FH10. **F.** Mean (±SEM) choice trials completed for fixed ratio schedules offered concurrently with FH10. Bonferroni correction; *, $p < 0.05$; **, $p < 0.01$.

*Appendix B*

***Post hoc* analyses of results**

**Figure 1. Choice behavior of subjects offered concurrent FR and FH work alternatives on the CEC task.**

**A.** Post-hoc analysis revealed a significant difference in the proportion of hold choices between 5 and 10 second hold requirements at ratio requirements FR01 ($p < 0.0001$), FR05 ($p = 0.0005$), FR10 ($p = 0.0006$), FR20 ($p < 0.0001$), FR40 ($p < 0.0001$) and FR80 ($p = 0.0208$).

**B.** There was a significant difference in the point of subjective equality between fixed hold requirements five and ten seconds ($t_{(15)} = 5.143$; $p = 0.0001$).

**C.** Post-hoc analysis revealed a significant difference in presses made between 5 and 10 second hold requirements at ratio requirements FR20 ($p = 0.0038$), FR40 ($p = 0.0001$), FR80 ($p < 0.0001$), and FR160 ($p = 0.0042$).

**D.** There was a significant difference in hold efficiency between FH05 and FH10 requirements at all ratio requirements ($p < 0.01$).

**E.** Post-hoc analysis revealed a significant difference in opt-out number between five and ten-second hold requirements when the ratio requirement was FR160 ($p < 0.0001$).

**Figure 2. Choice behavior of subjects offered concurrent FR schedules for pellet and dipper reward alternatives on the CVC task.**

**A.** There were significant differences in the proportion of rewards earned as dippers between concentrations of sucrose in the dipper at FR20 ($p = 0.0224$), FR40 ($p < 0.0001$), and FR80 ($p < 0.0001$).

**B.** There was a significant difference in the point of subjective equality, or the number of pellet presses subjects would equivocate with five presses for a dipper between sucrose concentrations ($t_{(15)} = 4.753$; $p = 0.0003$).

**Figure 3. The effects of the D2R antagonist Haloperidol on behavior in the CEC task.**

**A.** Post-hoc analysis revealed no significant differences in proportion hold choices between the vehicle and 0.1 mg/kg dose of haloperidol across ratio requirements. There was a significant difference between vehicle and 0.2 mg/kg dose of haloperidol at FR10 ($p = 0.0008$) and FR20 ($p = 0.0039$).

**B.** Analysis of the point of subjective equality, or the approximated number of presses equal to the 10-second hold requirement, revealed significant differences between treatment with vehicle and 0.1 mg/kg Haloperidol ($p = 0.0001$) and between vehicle and 0.2 mg/kg Haloperidol ($p = 0.0146$).

**C.** Post-hoc analysis revealed a significant difference in the number of responses made on the ratio bar between vehicle and the 0.1 mg/kg dose at FR40 ($p = 0.0442$) and FR80 ($p = 0.0026$). There were significant differences in presses between vehicle and 0.2 mg/kg of haloperidol at FR20 ($p = 0.0070$), FR40 ($p < 0.0001$), and FR80 ($p < 0.0001$). There were no significant differences in presses between 0.1 mg/kg and 0.2 mg/kg doses across ratio requirements.

**D.** Post hoc analysis of the hold efficiency across ratio requirements revealed significant differences between vehicle treatment and treatment with 0.2 mg/kg Haloperidol as well as treatment with 0.1 mg/kg Haloperidol and 0.2 mg/kg Haloperidol at FR10 ($p < 0.0001$). There was also a significant difference in hold efficiency between vehicle and 0.1 mg/kg Haloperidol at FR20 ($p = 0.0374$).

**E.** Post-hoc analysis revealed significant differences in opt outs taken between vehicle and the 0.1 mg/kg dose between vehicle and 0.2 mg/kg of haloperidol at all fixed ratio schedules ($p < 0.0001$), and between 0.1 mg/kg of haloperidol and 0.2 mg/kg of

haloperidol doses at all fixed ratio schedules of reinforcement ($p < 0.0001$). There were significant differences between treatment with vehicle and 0.1 mg/kg Haloperidol in opt outs taken when the ratio requirements were high (FR40; $p = 0.0411$; FR80; $p < 0.0001$).

**F.** Post-hoc analysis revealed no significant differences in the number of choice trials between vehicle and the 0.1 mg/kg dose across ratio requirements. There were significant differences in the number of choice trials completed between vehicle and 0.2 mg/kg of haloperidol at FR20, FR40, and FR80 ($p < 0.0001$), and between 0.1 mg/kg of haloperidol and 0.2 mg/kg of haloperidol doses at FR20, FR40, and FR80 schedules ($p < 0.0001$).

**Figure 4. The effects of the D2R antagonist Haloperidol on choice behavior in the CVC task.**

**A.** Post hoc analysis revealed significant differences in the proportion of rewards earned as a dipper when animals were offered a 5% sucrose solution and treated with vehicle versus 0.1 mg/kg Haloperidol at FR40 and FR80 ($p = 0.0065$; 0.0308). There was also a significant difference in choice behavior between when being treated with vehicle and given 5% sucrose solution versus 20% sucrose solution at FR10, FR20, FR40, and FR80 ($p = 0.0003$; 0.0003; $< 0.0001$; $< 0.0001$). Significant differences in choice arose between when subjects were offered 5% sucrose solution and treated with vehicle as compared to 20% sucrose and treated with haloperidol across all ratio requirements ($p = 0.0002$; $< 0.0001$; $< 0.0001$; $< 0.0001$). Differences between when subjects were offered 5% sucrose and treated with 0.1 mg/kg haloperidol and when they were offered 20% sucrose and injected with vehicle arose at FR40 and FR80 ($p = 0.0062$; $< 0.0001$). There was a significant difference in choice between the 5% sucrose – haloperidol condition versus the 20% sucrose – haloperidol condition at all pellet ratio requirements ($p = 0.0421$; $p <$

0.0001; < 0.0001; < 0.0001), and there was only a significant difference between 20% sucrose concentration treatments at FR20 (p = 0.0007).

**B.** There were no significant differences between drug treatments and sucrose concentrations on the point of subjective equality measure.

**Figure 5. The effects of striatal D2R overexpression on choice behavior in the CEC task.**

**A.** There were significant differences in the proportion of rewards earned using the hold lever between D2R-OEs on chow and all other groups at FR05 (controls on chow, p = 0.0106; controls on DOX, p = 0.0045; D2R-OEs on DOX, p = 0.0074), FR10 (p = 0.0026; 0.0009; 0.0037), FR20 (p < 0.0001; < 0.0001; = 0.0001), FR40 (p < 0.0001; < 0.0001; = 0.0001), and FR80 (p < 0.0001; < 0.0001; = 0.0009). There was also a significant difference between D2R-OEs on chow and controls on DOX at the highest requirement, FR160 (p = 0.0028). Post hoc analysis also revealed significant differences between controls on DOX and D2R-OEs on DOX at FR40 (p = 0.0030) and FR80 (p = 0.0013).

**B.** There was a significant difference between the points of subjective equality between D2R-OEs on chow and both controls on chow and those on DOX when the hold requirement was FH10 (p = 0.0416; 0.0183) and FH20 (p < 0.0001). There was also a significant difference between D2R-OEs on chow and those on DOX when the hold requirement was FH20 (p = 0.0048). There was also a significant difference in PSE when the hold requirement was 20 seconds between controls on DOX and D2R-OEs on DOX (p = 0.0088).

**C.** Within the control-chow group, there were significant differences in the proportion of rewards earned holding between FH05 and FH10 at FR05 and FR20 (p = 0.0388;

0.0113). There were significant differences between FH05 and FH20 at FR20, FR40, and FR80 ($p < 0.0001$). Post hoc analysis revealed significant differences in the proportion hold choice between FH10 and FH20 at FR40 and FR80 ($p < 0.0001$).

**D.** Post hoc analysis of choice behavior within the control DOX group revealed significant differences in the proportion of rewards earned holding between FH05 and FH10 at FR20 ($p = 0.0003$). There were significant differences between FH05 and FH20 at FR05, FR20, FR40, and FR80 ($p = 0.0116$; $< 0.0001$; $< 0.0001$; $< 0.0001$). Post hoc analysis revealed significant differences in the proportion hold choice between FH10 and FH20 at FR40 and FR80 ($p < 0.0001$).

**E.** The D2R-OE chow group showed no significant difference in proportion hold choices across all ratio requirements between FH05 and FH10. There was a significant difference in choice behavior between FH05 and FH20 at FR10 ($p = 0.0035$) as well as between FH10 and FH20 at FR20 ($p < 0.0001$).

**F.** D2R-OE subjects on DOX also showed no significant differences in choice behavior across ratio requirements between FH05 and FH10. There was a significant difference in choice between FH05 and FH20 at FR20, FR40, and FR80 ($p = 0.0002$; $< 0.0001$; $< 0.0001$). The proportion of hold choices made when the hold requirement was FH10 versus FH20 was significantly different at FR40 and FR80 ($p = 0.0011$; $< 0.0001$).

**Figure 6. The effects of striatal D2R overexpression on behavior in the CEC task.**

**A.** Post hoc analysis revealed significant differences in the number of responses made on the ratio lever between controls on chow and D2R-OEs on chow at FR40 ($p = 0.0047$), FR80 ($p = 0.0001$), and FR160 ($p = 0.0020$). There were also significant differences between controls on DOX and D2R-OEs on chow at FR40 ($p = 0.0001$), FR80 ($p < 0.0001$), and

FR160 ($p = 0.0007$). There were also differences in presses between controls on DOX and D2R-OEs on DOX at FR80 ($p = 0.0439$) as well as a difference between D2R-OEs on chow and those on DOX at FR80 ($p = 0.0012$).

**B.** Post hoc analysis revealed significant differences in the hold efficiency of groups across FR requirements between D2R-OEs on chow and all other groups at FR05 (all groups, $p < 0.0001$), FR10 ($p < 0.0001$), and FR20 (control chow and control DOX, $p < 0.0001$; D2R-OE DOX, $p = 0.0085$). There were significant differences in the hold efficiency between D2R-OEs on chow and control groups at FR40 (chow, $p = 0.0004$; DOX, $p < 0.0001$). There were significant differences in hold efficiency between D2R-OEs on chow and controls on DOX at FR80 ($p = 0.0085$) and FR160 ($p = 0.0149$).

**C.** There were significant differences between the control groups (controls on chow and controls on DOX) at FR40 ($p = 0.0004$; 0.0262, respectively) and FR80 ($p = 0.0004$), and there was also a significant difference between controls on chow and D2R-OEs on DOX at FR80 ($p = 0.0398$).

**D.** There was a significant difference between controls on chow and controls on DOX on the number of choice trials completed on the fixed ratio 160 schedule when the hold requirement was FH20 ($p = 0.0006$).

**Figure 7. The effects of striatal D2R overexpression on choice behavior in the CVC task.**

**A.** Post hoc analysis revealed no significant differences between groups on the proportion of rewards earned as a dipper when the concentration of sucrose in the dipper reward was 20%.

**B.** Post hoc analysis revealed significant differences in the point of subjective equality between controls on chow and all other groups when the concentration of sucrose in the

dipper was 5% (control DOX, p = 0.0024; D2R-OE chow, p = 0.0012; D2R-OE DOX, p = 0.0002).

**C.** Within the control group fed chow, there were significant differences in the proportion of dipper choices between sucrose concentrations across pellet ratio requirements (FR05, p = 0.0077; FR10, p = 0.0026; FR20, p = 0.0005; FR40, p < 0.0001; FR80, p < 0.0001).

**D.** The control group fed DOX maintained a significant difference in choice behavior between sucrose concentrations from pellet ratio requirement FR05 through FR40 (p = 0.0055; < 0.0001; < 0.0001; < 0.0001).

**E.** D2R-OEs fed chow only maintained significant differences in choice behavior between the two sucrose concentrations at FR05, FR10, and FR20 (p = 0.0027; p = 0.0064; p = 0.0122).

**F.** D2R-OEs treated with a DOX regimen also only maintained significant difference in choice behavior between the two sucrose concentrations from FR05 to FR20 (p = 0.0005; < 0.0001; < 0.0001).

**Figure 8. The effect of striatal D2R overexpression on behavior in the CEC task with a FH05 requirement alternative.**

**A.** Post hoc analysis revealed a significant difference in the proportion of rewards earned holding between D2R-OEs on chow and controls and D2R-OEs on DOX at FR10 (p < 0.0001), a difference between D2R-OEs on chow and controls on chow and DOX at FR20 (p = 0.0129; 0.0007), and a significant difference between controls on DOX and and D2R-OEs on chow and DOX at FR40 (p = 0.0148; 0.0212).

**B.** Post hoc revealed significant differences in the number of responses made on the ratio lever between D2R-OEs on chow and controls on chow and DOX as well as D2R-OEs on DOX at FR20 (p = 0.0003; < 0.0001; 0.0355) and FR40 (p < 0.0001; < 0.0001; =

0.0152). There was also a significant difference in the number of presses made between Controls on DOX and D2R-OEs on DOX at FR20 ($p = 0.0282$) and FR40 ($p < 0.0001$).

**C.** There were no significant differences between groups across ratio requirement on hold efficiency.

**D.** Post hoc analysis revealed a significant difference in the number of forced trials designated as opt outs between D2R-OEs on chow and controls on chow and DOX at FR20 ($p = 0.0233$; 0.0319) and significant differences between D2R-OEs on chow and controls on chow and DOX as well as D2R-OEs on DOX at FR40 ($p < 0.0001$; $< 0.0001$; $= 0.0002$) and FR80 ($p < 0.0001$).

**E.** Post hoc analysis revealed no significant differences between groups across ratio requirements in the number of choice trials they completed.

**Figure 9. The effect of striatal D2R overexpression on behavior in the CEC task with a FH10 requirement alternative.**

**A.** Analysis of the proportion of rewards earned by employing the FH10 requirement revealed significant differences between D2R-OEs and controls on chow and DOX as well as D2R-OEs on DOX at FR01 ($p = 0.0152$; 0.0079; 0.0123), FR10 ($p < 0.0001$), FR20 ($p < 0.0001$), and FR40 ($p < 0.0001$; $< 0.0001$, $= 0.0041$). At FR05, there was a significant difference between controls on chow and DOX and D2R-OEs on chow ($p = 0.0288$; 0.0159), and at FR20 and FR40, there emerged a significant difference between Controls on DOX and D2R-OEs on DOX ($p = 0.0331$; 0.0048)

**B.** Post hoc analysis revealed significant differences in presses between D2R-OEs fed chow and controls on chow and DOX, and D2R-OEs on DOX at FR20 ($p < 0.0001$; $< 0.0001$, $= 0.0005$) and FR40 ($p < 0.0001$; $< 0.0001$; $= 0.0021$). There was a significant difference between D2R-OEs on chow and controls on chow and DOX at FR80 ($p = 0.0029$;

0.0002). There also appeared a difference between controls on DOX and D2R-OEs on DOX at FR40 ($p = 0.0012$) and FR80 ($p = 0.0181$).

**C.** There were significant differences in the efficiency of subjects' holding between the D2R-OE on chow group and controls on chow, DOX, and D2R-OEs on DOX at FR01 ($p = 0.0440$; 0.0128; 0.0021), FR05 ($p = 0.0054$; 0.0002; 0.0013), FR10 ($p < 0.0001$), and FR20 ($p < 0.0001$; $< 0.0001$; $= 0.0046$). There was a significant difference between controls on DOX and D2R-OEs on chow at FR40 ($p = 0.0463$).

**D.** We found a significant difference in opt outs between D2R-OEs and controls on chow and DOX at FR40 ($p = 0.0004$; 0.0262) and FR80 ($p = 0.0004$; 0.0004). There was a significant difference between D2R-OEs on DOX and controls on chow at FR80 ($p = 0.0398$).

**E.** Post hoc analysis revealed no significant differences in the number of choice trials completed between groups across ratio requirements.