D2 DOPAMINE RECEPTOR MEDIATION OF RISKY DECISION-MAKING

A Dissertation

by

NICHOLAS WAYNE SIMON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2010

Major Subject: Psychology

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ABSTRACT

D2 Dopamine Receptor Mediation of Risky Decision-making. (May 2010)

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Excessive risk-taking is a characteristic of several psychopathological disorders. In order to alleviate maladaptive risky behavior, a thorough understanding of the neurobiological and pharmacological substrates of risky choice must be developed. In this dissertation, the “risky decision-making task” was utilized to explore the mechanisms by which dopamine mediates risky choice.

In experiment 1, we characterized rats in risky decision-making as well as a variety of other behavioral traits. This was performed to determine if the behavioral patterns obtained in the risky decision-making task represent an independent cognitive construct rather than a function of a separate behavioral trait. Risky decision-making performance was not correlated with measures of motivation, anxiety, pain tolerance, or other types of decision-making. In contrast, risky choice was correlated with impulsive action as assessed by the Differential Rates of Low Responding Task, suggesting that risky choice may be mechanistically similar to impulsive action.

In experiment 2, the effects of various dopaminergic drugs on risky decision-making was investigated. Amphetamine administration attenuated risky choice, while the dopamine antagonist α-flupenthixol had no effect on risky choice. Agonists and antagonists specific to D1 dopamine receptors had no effects on risky choice; however, the D2 dopamine receptor agonist bromocriptine reduced risky choice in a manner similar to amphetamine. Furthermore, co-administration of amphetamine with a D2 antagonist abolished amphetamine's effects on risky choice, and amphetamine's effects were unaffected by coadministration of a D1 antagonist. These data suggest that D2 signaling at the receptor is particularly critical to risky decision-making behavior.

In experiment 3, D2 dopamine receptor mRNA abundance was assessed in rats that had been previously characterized in risky decision-making using *in situ* hybridization. Levels of D2 cRNA hybridization in both orbitofrontal cortex (OFC) and medial prefrontal cortex (mPFC) predicted risky decision-making behavior as assessed by nonlinear curve estimation analyses. Interestingly, opposite relationships between D2 mRNA abundance and risky choice were observed in these two cortical areas, with OFC D2 mRNA abundance showing a U-shaped relationship with risky choice, and mPFC D2 mRNA resembling an inverted U-curve. Additionally, increased levels of D2 mRNA in dorsal striatum were observed in risk-averse rats in comparison to risk-taking rats. In conclusion, these data suggest that signaling via D2 dopamine receptors is an important mediator of risky decision-making behavior, and that D2 signaling in frontostriatal circuitry may be particularly relevant toward these behaviors.

DEDICATION

This dissertation is dedicated to my mother. Your strength is a constant inspiration to me. Thank you for everything you've taught and given me. Any creativity, expressive writing, or abstract thinking ability that I possess comes from you.

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INTRODUCTION

Throughout each day, people are faced with situations that require quick and effective decisions. The majority of these decisions require the careful assessment of an array of outcomes before determining which option is the most beneficial. Often, a subjectively favorable outcome is accompanied by some degree of risk. For example, exceeding the speed limit can confer benefits, such as arriving at one's destination sooner. However, there is also probability that this behavior will result in a citation for speeding that varies based on time of day, neighborhood, weather, or other factors. In order to make optimal decisions, people must be aware of surrounding contingencies and adjust their behavior accordingly. An inability to appropriately assess risky situations can lead to social problems, financial instability, and injury.

Individuals with various psychopathological and somatic disorders such as ADHD, schizophrenia, major depressive disorder, drug addiction, and Parkinson's disease demonstrate maladaptive risky decision-making (Bechara et al 2001; Drechsler et al. 2008; Ernst et al. 2003; Heerey et al. 2008; Kobayakawa et al. 2008; Ludewig et al. 2003; Taylor Tavares et al. 2007). A common pathology underlying most if not all of these disorders is abnormal dopamine transmission. In addition, healthy individuals subjected to dopamine depletion demonstrate increased risky choice (Sevy et al. 2006). Therefore, an understanding of the specific method by which dopamine mediates risky

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This dissertation follows the style of *Annual Review of Neuroscience.*

decision-making is important for the treatment of excessive risk-taking. The development of an animal model that is both reliable and possesses face validity as a model of complex risky decisions is critical for the delineation of the pharmacological and neural substrates involved with risky choice.

Unfortunately, although there are several reliable models of animal decision-making that have been well-characterized (for reviews see Cardinal 2006; Floresco et al. 2008), there are few that integrate rewarding outcomes with the risk of a punishing stimulus. To fill this void, we developed a task in which rats choose between a small, safe food reward and a large, risky food reward that is associated with a systematically increasing probability of punishment (footshock). It was observed that rats performing this "risky decision-making" task display a shift in behavioral preference from the risky to safe reward as the risk of punishment increases, and that performance in this task remains reliable over long periods of time (Simon et al. 2009). Interestingly, a trend that persisted throughout several cohorts of rats was the presence of a subset of rats that can be characterized as "risk-taking", showing an almost 100% preference for the risky reward. This phenotype could prove exceptionally useful as a model of pathological risk-taking.

However, the utility of the risky decision-making task is contingent upon the construct of "risk" existing as an independent trait rather than as an artifact of a separate behavioral construct or an aggregate of various behavioral factors. In other words, rats' willingness to risk punishment as assessed by the risky decision-making task could simply be governed by a combination of behavioral

constructs such as pain tolerance and reward motivation. Experiment 1 of this dissertation addressed this concern by characterizing rats on the risky decision-making task, then testing the same rats on measures of several additional behavioral constructs that may influence or share variance with risky decision-making performance. Behavior was measured in three other cost-benefit decision-making tasks: probabilistic discounting, which measured the degree to which risk of reward omission (rather than physical punishment) discounts reward value (St Onge & Floresco 2009); delay discounting, which measured willingness to tolerate delayed rewards (Evenden & Ryan 1996), and effort-based discounting, which measured rats' willingness to exert effort for large rewards (Ghods-Sharifi et al. 2009). Several other traits that may influence risky decision-making performance were also assessed, including multiple measures of motivation, pain sensitivity, and impulsive action (the inability to withhold a prepotent motor response). The relationships between these data and risky decision-making performance as well as with performance on the other cost-benefit decision-making tasks were then analyzed.

Experiment 2 consisted of a series of pharmacological experiments designed to test the influence of acute administration of various dopaminergic agents on risky decision-making. Acute systemic injections of amphetamine, flupenthixol (a non-specific dopamine receptor antagonist) and D1 and D2 dopamine receptor specific agonists and antagonists were all administered prior to testing. There is prior evidence that amphetamine decreases risky decision-making (Simon et al. 2009); in order to delineate the dopamine receptor subtype

that specifically mediates the effects of amphetamine, amphetamine was co-administered with antagonists specific for each receptor subtype.

Experiment 3 tested the hypothesis that baseline differences in dopamine receptor level account for some of the variability observed in risky decision-making. This was achieved by performing *in-situ* hybridization to measure mRNA for the specific dopamine receptor subtype (D2) found to mediate risky decision-making in Experiment 2. The untreated rats previously characterized in risky decision-making as well as the other cost-benefit decision-making tasks and behavioral control measures (Experiment 1) were used for this experiment to allow the comparison between multiple behavioral measures and dopamine receptor mRNA. *In-situ* hybridization offered the advantage of brain region-specific analysis; the areas in which mRNA was quantified were the orbitofrontal cortex, medial prefrontal cortex, dorsal striatum, and nucleus accumbens, all brain regions shown previously to contribute to reward- and punishment- related decision-making (Bechara et al. 2000; Cardinal 2006; Clark et al. 2008; Morrison & Salzman 2009; Naqvi & Bechara 2009; Roesch et al. 2007a; St. Onge & Floresco 2009; Winstanley et al. 2004b; Winstanley et al. 2006).

METHODS

Experiment 1: The Relationship between Risky Decision-making and Other Behavioral Measures

Subjects

Male Long-Evans rats (n=18; Charles River Laboratories, Raleigh, NC weighing 275-300 g upon arrival) were individually housed and kept on a 12 hour light/dark cycle (lights on at 0800) with free access to food and water except as noted. All procedures were conducted in accordance with the Texas A&M University Laboratory Animal Care and Use Committee and NIH guidelines.

Behavioral Testing

Decision-making Tasks

Risky Decision-making Task

Shaping procedures were identical to those used previously (Simon et al. 2007). Following magazine training, rats were trained to press a single lever (either left or right, counterbalanced across groups; with the other retracted during this phase of training) to receive a single food pellet. After reaching a criterion of 50 lever presses in 30 minutes, rats were shaped to press the opposite lever under the same criterion. This was followed by further shaping sessions in which both levers were retracted and rats were shaped to nose-poke into the food trough during simultaneous illumination of the trough and house lights. When a nose-poke occurred, a single lever was extended (left or right), and a lever press resulted in immediate delivery of a single food pellet. Immediately following the lever press, the house and trough lights were

extinguished and the lever was retracted. Rats were then trained to a criterion of at least 30 presses of each lever in 60 minutes. This shaping procedure was sufficient for all three decision-making tasks.

Test sessions were 60 minutes long and consisted of five blocks of 18 trials each. Each 40 s trial began with a 10 s illumination of the food trough and house lights. A nose poke into the food trough during this time extinguished the food trough light and triggered extension of either a single lever (forced choice trials) or of both levers simultaneously (choice trials). If the rats failed to nosepoke within the 10 s time window, the lights was extinguished and the trial scored as an omission.

A press on one lever (either left or right, balanced across animals) resulted in one food pellet (the small, safe reward) delivered immediately following the lever press. A press on the other lever resulted in delivery of three food pellets (the large reward). However, selection of this lever was also accompanied immediately by a possible 1 s footshock contingent on a preset probability specific to each trial block. The probability of footshock accompanying the large reward was set at 0% during the first 18-trial block. In subsequent 18-trial blocks, the probability of footshock increased to 25, 50, 75, and 100%. Each 18-trial block began with 8 forced choice trials used to establish the punishment contingencies (4 for each lever), followed by 10 choice trials (Cardinal & Howes 2005; St Onge & Floresco 2009). Once either lever was pressed, both levers were immediately retracted. Food delivery was accompanied by re-illumination of

both the food trough and house lights, which was extinguished upon entry to the food trough to collect the food or after 10 s, whichever occurred sooner.

Locomotor activity was assessed during each shock presentation and averaged across the entire session for each subject. This measure of shock reactivity was utilized as an assessment of pain tolerance to be compared with the other tasks.

Probabilistic Discounting

The parameters of this task were identical to the risky decision-making task, with the main difference following selection of the large reward lever. During the first block of trials, the large reward was delivered with 100% probability. During each of the four subsequent blocks, the probability of large reward delivery was systematically decreased (50, 25, 12.5, 0%). The large reward was accompanied by neither punishment nor a delay period. Each block was preceded by 8 forced choice trials with equal random presentations of each lever, and each trial lasted for 40 seconds. Each full session lasted 60 minutes.

Delay Discounting

For a detailed version of this task methodology, see Simon et al. (2007). Delay discounting task design resembled the risky decision-making task, with the critical difference being the reward delivery associated with the large reward lever. Selection of this lever again resulted in 3 food pellets, but during this task, reward delivery was preceded by a delay (with no risk of footshock). The delay escalated with each 10-trial block (0, 4, 8, 16, and 32 s). Each trial lasted 60 seconds, and each session lasted 60 minutes.

Effort-based Discounting

This task was modified from Floresco et al. (2007). The basic parameters were similar to the previous decision-making tasks, with exceptions for reward size and the criterion required to obtain the large reward. A lever press on the small reward lever caused delivery of 2 food pellets immediately. Selection of the large reward lever caused the small reward lever to retract and the large reward lever to remain extended; from that point, multiple lever presses were required to achieve fulfillment of an effort-based criteria. After the criterion was met, both levers were retracted and 4 food pellets were delivered. The effort criteria for the 5 blocks were 1, 2, 4, 8, and 16 lever presses. Each session lasted 60 minutes.

Motivation Assessment Tasks

Sucrose Consumption

Rats were given access to daily 30-min tests for separate concentrations of sucrose solutions (0, 2.5, 5, 10, 20% in tap water; counterbalanced order) while in their home cages. Sucrose consumption was measured by weighing each sucrose container before and after each test session. This measure was used as a measure of reward motivation.

Fixed Ratio and Progressive Ratio Responding

Rats were again food restricted to 85% of their free feeding weight prior to testing. Motivation for food reward was assessed daily using 30 minute fixed ratio (FR) schedules (FR1, 3, 10, 20, 40). Following FR testing, motivation was assessed further using a progressive ratio schedule of reinforcement, on which the number of lever presses required to earn a reward increased with each

successive reward earned (1, 4, 10, 20, 35, …) (Mendez et al. 2009). These sessions varied in length, ending only after an hour with no reward delivery had passed (the breakpoint).

Pain Tolerance Assessment Tasks

Shock Locomotion

This measure of shock reactivity was acquired during performance of the risky decision-making task. During the 1-s footshock that followed selection of the large, risky reward, overall locomotor activity was measured, and an average of these scores was used as a measure of shock sensitivity (Chhatwal et al. 2005; Simon et al. 2009).

Tail Flick Test

An IITC Model 33A tail-flick apparatus were used as a measure of pain tolerance (Mendez & Trujillo 2008). This device focused a hot lamp on the tail of the animal, between 2 and 8 centimeters from the tip. The amount of time before each subject moved its tail from the heat was automatically recorded. A heat setting that yielded a 3-6 second baseline response was used, and an automatic cut off time of 10 seconds was set to avoid any tissue damage. Tail flick latency was determined by taking the mean of three tail flicks, separated by 15-20 seconds. These data were used as a measure of pain sensitivity.

Shock Sensitivity Testing

This procedure was modified from King et al. (1996). First, rats were restrained in a plexiglass tube and habituated for 15 minutes. Shock sensitivity was assessed using a manual shocker (BRS/LVE, Model SG-903) that allowed

continuous variation of shock intensity between 0 and 2 mA. Test shocks were applied 7 cm from the base of the tail through electrodes constructed from lightweight fuse clips. Shock intensity was gradually increased at a rate of .05 mA every 3 seconds. Latency to movement and vocalization were then assessed, after which the shock was terminated.

Anxiety-Assessment Tasks

Elevated Plus Maze

The elevated (73 cm from the floor) plus-maze (EPM) consisted of two opposing closed arms and two opposing open arms (42.7 cm length × 15.2 cm width/arm; arm enclosure height: 22.9 cm), and a central platform. Each 10-min test period began with the rat facing the left open arm, with behavior recorded using a camera suspended over the maze. Following testing, the amount of time spent in the open arms and the amount of open arm entries was scored manually in order to formulate an anxiogenic profile for each subject (the amount of time spent in/entries into open arms was characterized as inversely related to general anxiety) (Schulteis et al. 1998; Wingard & Packard 2008). Total open and closed arm crossovers were also tabulated as a measure of general activity.

Locomotion Test

Baseline locomotion and overall exploratory behavior were tested in activity monitoring chambers (Versamax System, Accuscan Instruments, Columbus, Ohio). Each chamber (40 x 40 x 30 cm) contained an array of photobeams used to detect movement in the horizontal plane throughout one hour-long session.

Impulsive Action Assessment Tasks

Differential Reinforcement of Low Rates of Responding Task (DRL)

The DRL has been utilized as a measure of impulsive action, defined as the inability to withhold a prepotent motor response (Sokolowski & Salamone 1994; Uslaner & Robinson 2006). Each DRL session was 45 minutes in length. Rats were trained on DRL-5s schedules for five days, during which a lever press only resulted in food pellet delivery if at least five seconds had elapsed since the previous press. Then, rats were trained for five days each on DRL-10s and DRL-20s schedules. Finally, rats were given 15 days of training in a DRL-30s schedule. Impulsive action was assessed on day 15 as the ratio of unrewarded responses (lever presses during the 30 seconds after the previous, rewarded press) to total responses.

Overall Experimental Timeline

Risky decision-making (25 days) - Elevated plus maze (1 day) - Tail flick (1 day) - Locomotion (1 day) - Sucrose consumption (5 days) - Fixed ratio responding (5 days) - Progressive ratio (1 day) - DRL(30 days) – Probabilistic discounting (20 days) – Delay discounting (10 days) – Effort-based discounting (10 days) – Rebaseline risky decision-making (10 days) – Shock sensitivity testing (1 day)

Experiment 2: The Effects of Dopaminergic Manipulation on Risky Decision-making

Subjects

A group of 12 male Long-Evans rats were used for amphetamine and flupenthixol treatment. A separate group of 12 male Long-Evans rats were used for all other treatment conditions.

Drugs

The compound d-Amphetamine sulfate (Sigma, St. Louis MO; .33, 1.0, 1.5 mg/kg) was selected as a dopamine enhancing drug, and α-flupenthixol (Sigma, .125, .25, .5 mg/kg) was used as a non-specific dopaminergic antagonist. SKF81297 (Tocris Bioservices, Ellisville, MO; 0.1, .3, 1.0 mg/kg) and SCH23390 hydrobromide (Tocris; .005, .01, .03 mg/kg) were used as D1-specific agonist and antagonists, respectively. Bromocriptine mesylate (Tocris; 1.0, 3.0, 5.0 mg/kg) was used as a D2-specific agonist, and eticlopride hydrochloride (Tocris; .01, .03, .05 mg/kg) was used as a D2 antagonist. All drugs were dissolved in 0.9% saline vehicle expect the D2 agonist Bromocriptine, which was dissolved in dimethyl sulfoxide and then diluted at a 50:50 ratio with saline. Amphetamine and SKF81297 were administered 10 minutes prior to testing, SCH23390 and eticlopride were administered 20 minutes prior to testing, and bromocriptine was administered 40 minutes prior to testing (based on St. Onge et al., 2009).

Experimental Procedure

Each drug treatment was administered on an eight day schedule, with injections on days 1, 3, 5, and 7. The three doses of drug and a saline treatment

were counterbalanced across these four days. There were no treatments on days 2, 4, 6, and 8; these baseline days were used to confirm an absence of baseline shifts in behavior. Prior to each drug treatment regimen, rats were given a minimum of five days of baseline testing to ensure stable performance.

Experiment 3: The Relationship between Dopamine Receptor mRNA and Cost-benefit Decision-making

Subjects

The same group of untreated, behaviorally characterized rats (n=18) utilized for Experiment 1 were used for this experiment.

Tissue Preparation

Rats were sacrificed with a 100 mg/kg sodium pentobarbital solution, then perfused with 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde solution overnight, then post-fixed in 4% paraformaldehyde 20% sucrose on the following day. The brains were sectioned (30 μm thickness) on the coronal plane, and were collected in a 1-6 series beginning at the anterior portion of prefrontal cortex (5.2mm Bregma), and ending posterior to the nucleus accumbens (-0.26 mm Bregma). For analyses, prefrontal cortex was divided into two subregions: orbitofrontal cortex (OFC),encompassing the orbitofrontal and insular cortices, and medial prefrontal cortex (mPFC), including infralimbic, prelimbic, and anterior cingulate cortex. The dorsal striatum (DS) and nucleus accumbens (NAcc) were also analyzed separately.

Probe Preparation

The D2 cRNA probe consisted of 331 basepairs corresponding to basepairs atg (bps 416-18) through tga (bps 1748-50) of the full D2 receptor transcript. The cRNA probe was separated using an antisense T7 RNA polymerase transcribed in the presence of ^{35}S-labelled UTP.

In Situ Hybridization

Free-floating sections of tissue were washed in 0.75% glycine in 0.1 M phosphate buffer,pH 7.2 (PB) and 0.1 M PB alone to remove excess fixative. Sections were treated for 30 min at 37 °C with proteinase K (1 mg/mL in 0.1 M Tris buffer containing 0.05% SDS), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, and rinsed twice in 2× saline sodium citrate buffer (SSC; 1 × SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0). Tissue was then hybridized for 42–44 h at 60 °C in solution containing 50% formamide, 1 × Denhardt's solution, 10% dextran sulphate, 4 × SSC, 0.25 mg/mL yeast tRNA, 0.3 mg/mL herring sperm DNA, 100 mm dithiothreitol (DTT) and the ^{35}S-labelled D2 cRNA at a final concentration of 1×10^7 CPM/mL. Following hybridization, sections were washed at 30 min intervals, twice in 4 × SSC, once in 50% formamide/2 × SSC at 60 °C and then treated with ribonuclease A (20 mg/mL in 10 mM Tris saline buffer containing 1 mM ethylene-diaminetetracetic acid) for 30 min at 37 °C. Tissue sections were then washed further in descending concentrations of SSC buffer containing 100 mM DTT to a final wash of 0.1 × SSC and mounted onto gelatin-coated slides for film autoradiography. Air-dried sections of the sections were exposed along with ^{14}C-

standards to phosphoimage screens (Perkin Elmer, Waltham MA). Because D2 dopamine receptor mRNA is less abundant in prefrontal cortex than in striatum, brain sections containing prefrontal cortex were exposed for 72 hours, while the tissue containing DS or NAcc were exposed for 24 hours. Screens were scanned at high resolution using a Typhoon Phosphoimager (Perkin Elmer, Waltham MA).

Relative D2 mRNA abundance was quantified by densitometric analysis using Densita imaging software (MBF Biosciences, Williston, VT). Hybridization densities were linearized and calibrated relative to the ^{14}C-labelled standards that were exposed to each phosphoscreen along with tissue sections. Multiple measures were obtained from 4-6 sections per brain region animal. For each brain structure analyzed, these values were averaged to provide an individual mean hybridization density (μCi/g protein) per region in each subject. These means were used from correlations and group comparisons.

Data Analysis

Experiment 1

Each decision-making task continued until behavior reached stability across a five-day period, as determined by a lack of a repeated measures ANOVA effect of day across those five days. Data presented represent averages of performances across this five-day period of stable performance. Performance measures included in the data analysis were as follows: percent choice of large reward for each task, baseline weight prior to testing, total locomotion during footshock presentation in the risky decision-making task (a measure of shock reactivity), and latency to initiate each trial. These measures were compared to

each other using Pearson's correlations. Decision-making data were also compared with data from the EPM, tail flick test, locomotion test, sucrose consumption tests, fixed and progressive ratio responding, DRL, and shock sensitivity to identify any relationships between individual rats' performance across tasks. The relationship between behavioral measures was further analyzed using multiple regressions to determine if clusters of variables were able to predict the decision-making measures. Because of the large number of variables being considered, the alpha level for all correlations and regressions was set at .01. This reduced the possibility of significant correlations occurring as a result of chance.

Experiment 2

Drug treatments were analyzed using repeated measures ANOVAs (drug dose X punishment probability). The baseline days (days 2, 4, 6, and 8 of each injection schedule) were compared with each other also using repeated measures ANOVAs; a lack of a repeated measures effect indicated stable behavior. Locomotion was measured with an automated locomotion tracker (Coulbourn Instruments).

Experiment 3

Hybridization of D2 dopamine receptor cRNA in prefrontal cortex, dorsal striatum, and nucleus accumbens were analyzed was quantified as hybridization signal intensity values using scales determined by exposing C^{14} standards along with the tissue. One subject was removed from all prefrontal cortex analyses because of excessive tissue damage.

Pearson's correlations were then run between hybridization signals and all behavioral scores. However, because dopaminergic mediation of complex behavioral tasks frequently follows a non-linear pattern that correlations would not detect (such as U or inverted U curve), additional analyses were performed on these data. First, nonlinear regressions were utilized to determine if there was a quadratic relationship between variables. Then, as an additional measure, rats were divided into three groups on the basis of behavioral characterization on each of the four decision-making tasks as well as impulsive action. Hybridization intensity was compared between these groups using one way ANOVAs. The other, less complex behavioral measures (motivation, anxiety, and pain tolerance) were only subjected to correlational analysis.

RESULTS

Experiment 1 Results

Decision-making Tasks

Risky Decision-making

Rats achieved stable responding on sessions 16-20. Rats demonstrated a repeated measures effect of punishment probability ($F_{(4,68)}$ = 21.15, p <.001), indicating that rats discounted the large reward as a function of punishment probability (Figure 1).

Probabilistic Discounting

Stable responding was achieved on sessions 16-20. Rats demonstrated a repeated measures effect of large reward probability ($F_{(4,68)}$ = 85.22, p <.001), indicating that rats discounted the large reward as a function of delivery probability (Figure 2).

Delay Discounting

Stable responding was reached on sessions 6-10. Rats demonstrated a repeated measures effect of delay duration ($F_{(4,68)}$ = 46.49, p <.001), such that rats discounted the large reward as a function of its delay (Figure 3).

Effort-based Discounting

Stable responding was reached on sessions 6-10. Rats demonstrated a repeated measures effect of effort ($F_{(4,64)}$ = 39.58, p <.001), such that rats

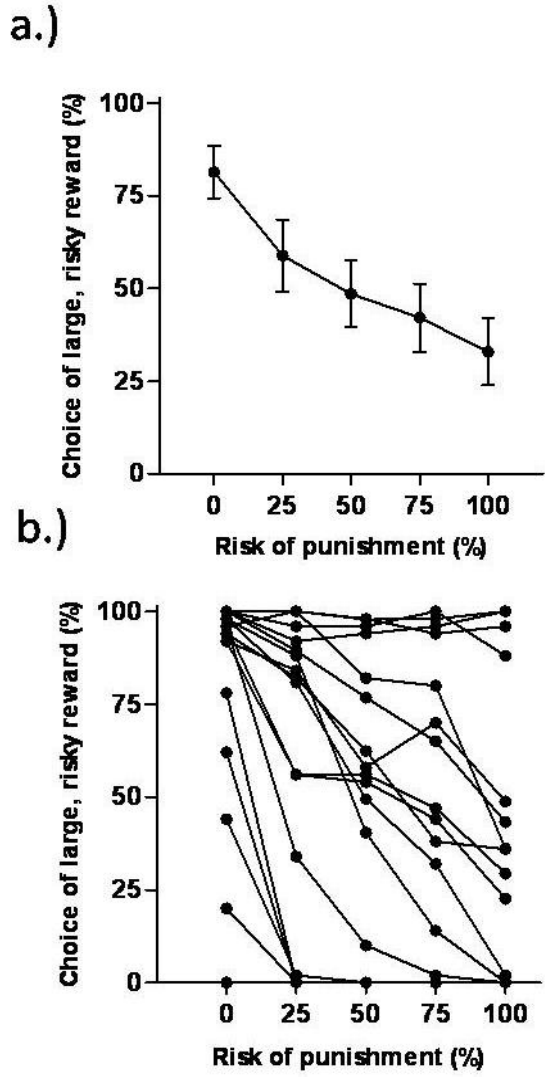


Figure 1: a.) Risky decision-making group mean. b.) Individual variability of risky decision-making scores.

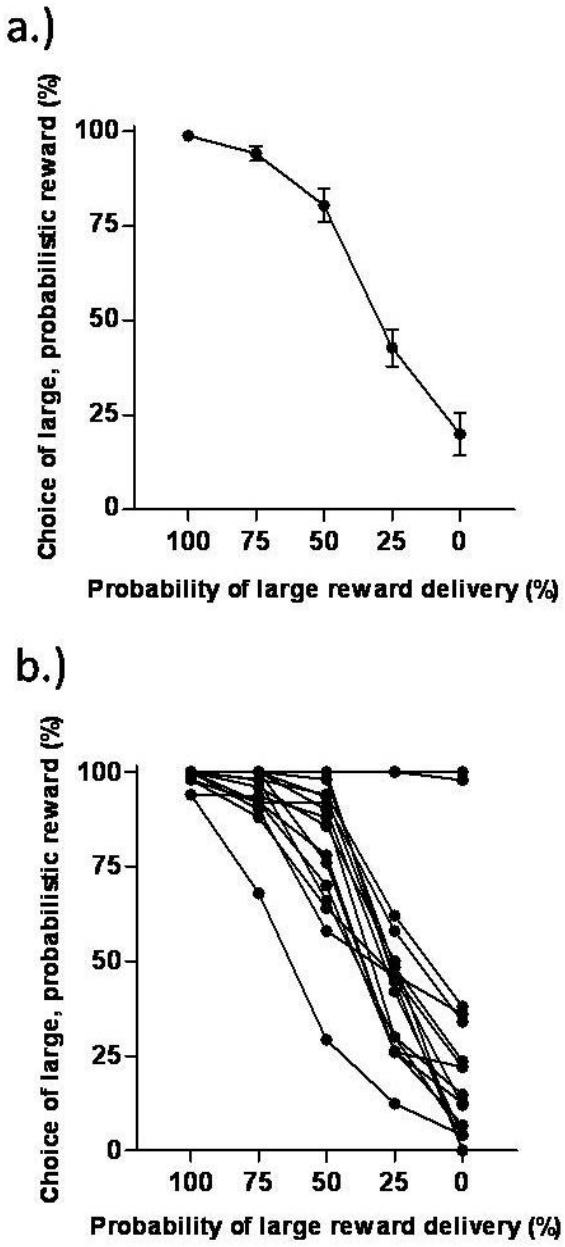


Figure 2: a.) Probabilistic discounting group mean. b.) Individual variability of probabilistic discounting scores

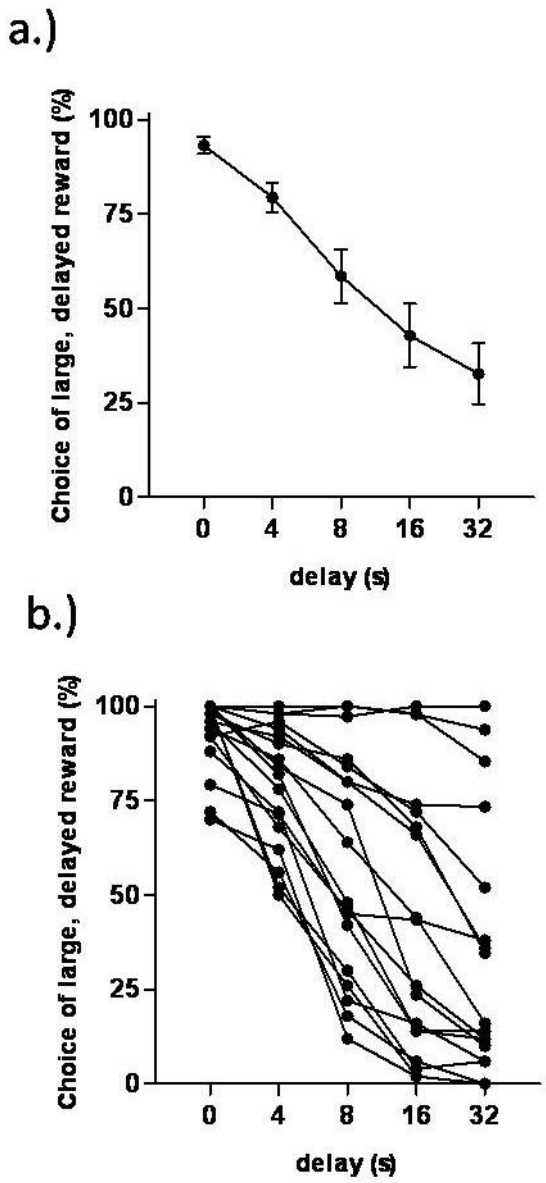


Figure 3: a.) Delay discounting group mean. b.) Individual variability of delay discounting scores

discounted the large reward as a function of the amount of effort required (Figure 4).

Comparisons of Performance across Decision-making Tasks

Pearson's r correlations between tasks are listed in Table 1. There was a significant positive correlation between performance on the delay discounting and effort-based discounting tasks (r = .60, p < .01), but there were no correlations between any other tasks (ps > .15).

Motivation Assessment Tasks

Instrumental Responding for Food Reward under Fixed and Progressive Ratios

Distributions and means for each fixed ratio (FR) and the progressive ratio schedule are displayed as Figure 5a. Lever presses on the FR1 schedule were not correlated with lever presses on any other FR schedule (rs < .36, *n.s.*). Performance on the FR3 schedule approached significant positive correlations with the FR10, FR20, and FR40 schedules (rs > .54, ps < .02), and performance was correlated between the FR10, FR20, and FR40 schedules (rs > .88, p <.001). Progressive ratio performance (as assessed by break point) was not correlated with lever presses on the FR1 schedule (r =.21, *n.s.*), was near a correlation with FR3 performance (r = .50, p = .03), and was correlated with FR10, FR20 and FR40 (rs > .76, ps < .001).

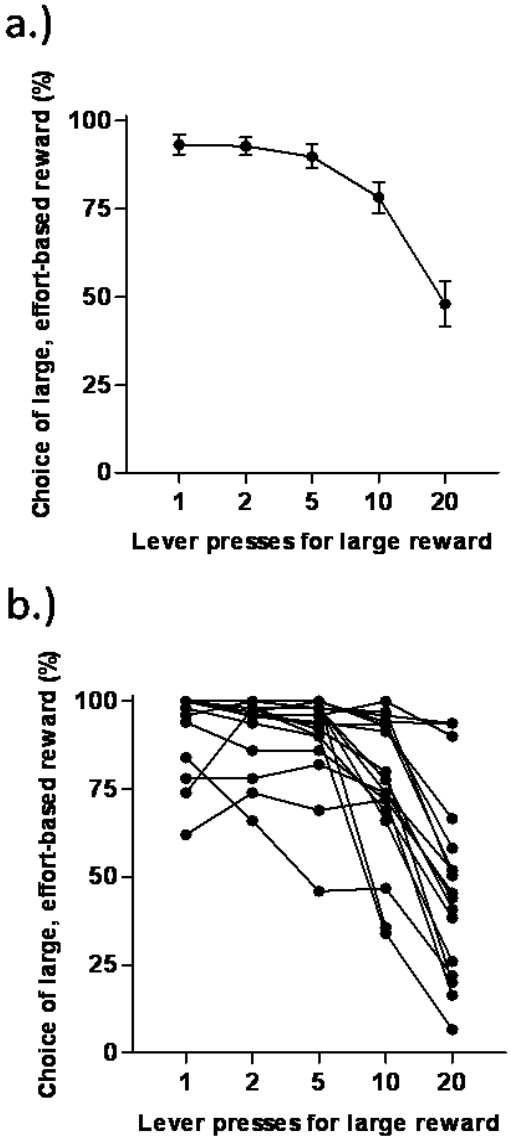


Figure 4: a.) Effort-based discounting group mean. b.) Individual variability of delay discounting scores

Table 1: Correlation matrix displaying relationships between cost-benefit decision-making tasks. There was a significant correlation between effort-based discounting and delay discounting. No other correlations were observed between tasks (α= .01).

	Risky Decision-Making	Delay Discounting	Probabilistic Discounting	Effort based Discounting
Risky Decision-Making	X	r = -.04 p = .88	r = -.13 p = .63	r = .27 p = .28
Delay Discounting	X	X	r = .36 p = .15	***r* = .60**** ***p* < .01**
Probabilistic Discounting	X	X	X	r = -.10 p = .70
Effort-based Discounting	X	X	X	X

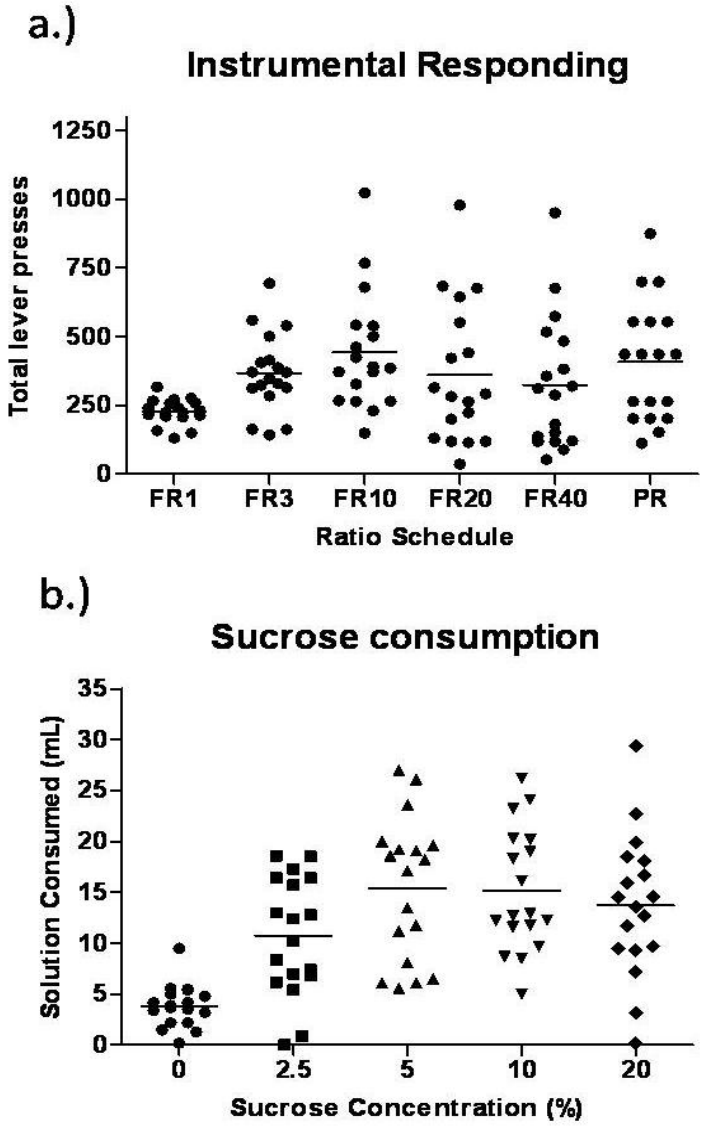


Figure 5: a.) Distribution of instrumental responding scores. b.) Distribution of sucrose consumption scores.

Sucrose Consumption

There was a repeated measures difference in consumption between the five doses of sucrose solution tested ($F_{(4,68)}$ = 23.2, *p.s* < .001) (Figure 5b). Individual analyses revealed that each of the solutions containing sucrose (2.5, 5, 10, and 20% solutions) were consumed to a greater extent than the 0% sucrose solution (*p*s < .001). Further analyses demonstrated that equal amounts of 5, 10, and 20% solution were consumed (*ps* >.26), and the amount of 5, 10, and 20% solutions consumed were each greater than the amount of 2.5% solution consumed (*ps* <.05). There were significant correlations within subjects for sucrose consumption at almost all concentrations (*ps* < .01), with the exceptions of the correlations between 5 and 10% and 2.5 and 20% sucrose (*ps* < .05), which approached significance.

Comparison of Performance between Motivation Assessment Tasks

Correlations between motivational measures are displayed in Table 2. Instrumental responding on a FR1 schedule was correlated with consumption of a 5% sucrose solution (*r* = .59, *p* = .01), and was near-correlated with consumption of 2.5 (p =.04) and 20% sucrose (*p* = .02). Multiple regression analyses were performed to determine if combinations of variables displayed any predictive potential within the motivation variables measures. Sucrose consumption variables did not predict performance on any of the instrumental responding schedules (*ps* > .12), nor did the instrumental responding variables predict sucrose consumption at any concentration (*ps* > .68).

Table 2: Correlation matrix displaying relationships between motivation assessment tasks. While several instrumental responding and sucrose consumption measures were correlated with each other, the only correlations that achieved significance between tasks were a positive relationship between FR1 with 5% sucrose and a negative relationship between FR10 with 5% sucrose (α= .01).

	FR1	FR3	FR10	FR20	FR40	PR	2.5% sucrose	5% sucrose	10% sucrose	20% sucrose
FR1	X	*r*= .36 *p*= .15	*r*= .044 *p*= .86	*r*= .36 *p*= .15	*r*= .19 *p*= .45	*r*= .23 *p*= .37	***r*= .49* *p*= .04**	***r*= .59** *p*= .01**	*r*= .46 *p*= .06	***r*= .54* *p*= .02**
FR3	X	X	***r*= .54* *p*= .02**	***r*= .56* *p*= .02**	***r*= .54* *p*= .02**	***r*= .54* *p*= ..02**	*r*= -.13 *p*= .61	*r*= -.06 *p*= .81	*r*= .0 *p*= .99	*r*= .14 *p*= .59
FR10	X	X	X	***r*= .90** *p*< .01**	***r*= .88** *p*< .01**	***r*= .78** *p*< .01**	*r*= -.38 *p*= .12	*r*= -.38 *p*= .01	*r*= -.21 *p*= .39	*r*= -.02 *p*= .93
FR20	X	X	X	X	***r*= .95** *p*< .01**	***r*= .85** *p*< .01**	*r*= -.30 *p*= .04	*r*= -.20 *p*= .42	*r*= -.27 *p*= .28	*r*= -.04 *p*= .89
FR40	X	X	X	X	X	***r*= .93** *p*< .01**	*r*= -.34 *p*= .17	*r*= -.10 *p*= .70	*r*= -.27 *p*= .27	*r*= .03 *p*= .89
PR	X	X	X	X	X	X	*r*= -.34 *p*= .17	*r*= .04 *p*= .88	*r*= -.18 *p*= .47	*r*= .05 *p*= .85
2.5% sucrose	X	X	X	X	X	X	X	***r*= .64** *p*< .01**	***r*= .60** *p*= .01**	***r*= .57** *p*= .01**
5% sucrose	X	X	X	X	X	X	X	X	***r*= .50* *p*= .04**	***r*= .06** *p*= .01**
10% sucrose	X	X	X	X	X	X	X	X	X	***r*= .59** *p*= .01**
20% sucrose	X	X	X	X	X	X	X	X	X	X

Pain Tolerance Assessment Tasks

Shock Locomotion

The distribution of individual shock locomotion scores (quantified as average locomotor units during 1-s shock presentations on the risky decision-making task) is displayed as Figure 6a.

Tail Flick Test

There was no difference between the three measures of tail flick obtained ($F_{(2,34)}$ = 1.13, *n.s*); therefore, an average of each subject's scores was used for subsequent analyses (Figure 6b).

Shock Sensitivity Testing

All shock sensitivity scores are displayed as Figure 6c. The shock intensity required to elicit a motor response was strongly correlated with the intensity that produced a vocal response (r =.91, p < .001).

Comparison between Measures of Pain Tolerance

There was a near significant correlation between shock locomotion and tail flick performance such that higher tail flick latencies were associated with increased shock locomotion (r =.58, p = .03). There were no correlations between other measures of pain tolerance (*ps* > .09) (Table 3). Additionally, a multiple regression using both measures of shock sensitivity to predict tail flick latency was not significant (p = .21).

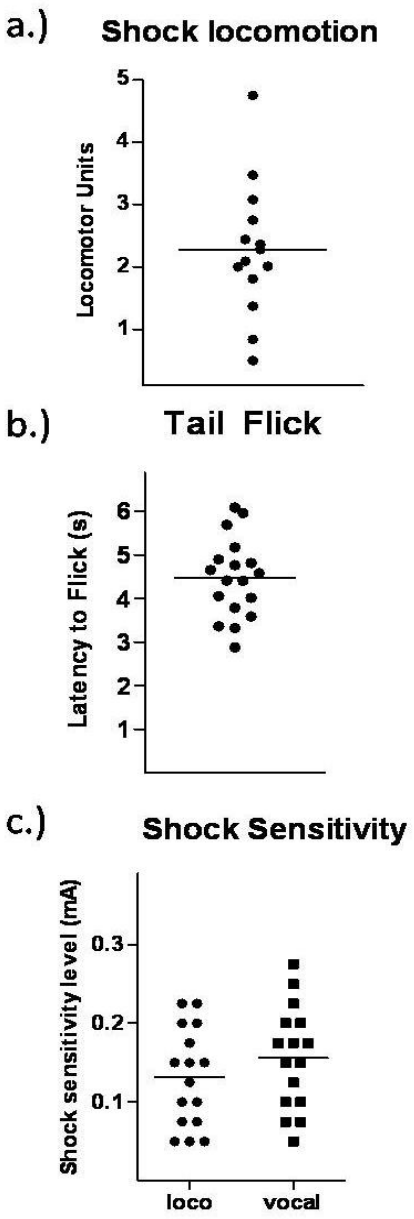


Figure 6: a.) Distribution of shock locomotion scores. b.) Distribution of tail flick latencies. c.) Distribution of both locomotor and vocalization shock threshold scores.

Table 3: Correlation matrix displaying relationships between pain sensitivity measures. The only significant relationship was a positive correlation between shock sensitivity threshold for vocalization and shock sensitivity threshold for motor activity (α= .01).

	Tail Flick Mean	Shock Locomotion	Shock Sensitivity: Motor	Shock Sensitivity: Vocal
Tail Flick Mean	X	***r* = .58*** ***p* = .03**	*r* = -.36 *p* = .17	*r* = -.44 *p* = .09
Shock Locomotion	X	X	*r* = -.27 *p* = .38	*r* = -.35 *p* < .24
Shock Sensitivity: Motor	X	X	X	***r* = .94**** ***p* < .01**
Shock Sensitivity: Vocal	X	X	X	X

Anxiety Assessment Tasks

Elevated Plus Maze

The distribution of time spent in the open arms and total open arm entries are displayed as Figure 7a. There was a significant correlation between open arm entries and time spent in open arms (r = .711, p < .01).

Locomotion Test

Horizontal activity and distance traveled in the locomotor activity chambers, both general measures of baseline activity, are displayed as Figure 7b. Time spent in the center of the chamber, commonly used as a measure of anxiety, is displayed as Figure 7c. Horizontal activity and distance traveled were positively correlated with each other (r = .96, p <.001), and neither of these measures were correlated with time in center (rs < .22, *n.s.*).

Comparison between Anxiety Measures

No correlations were observed between any measures of EPM and locomotion (ps > .46; Table 4). Multiple regression analyses revealed that a combination of all measures from the EPM did not predict any of the locomotor behaviors (ps > .47), nor did the combined measures of locomotion predict any measures from the EPM (ps > .59).

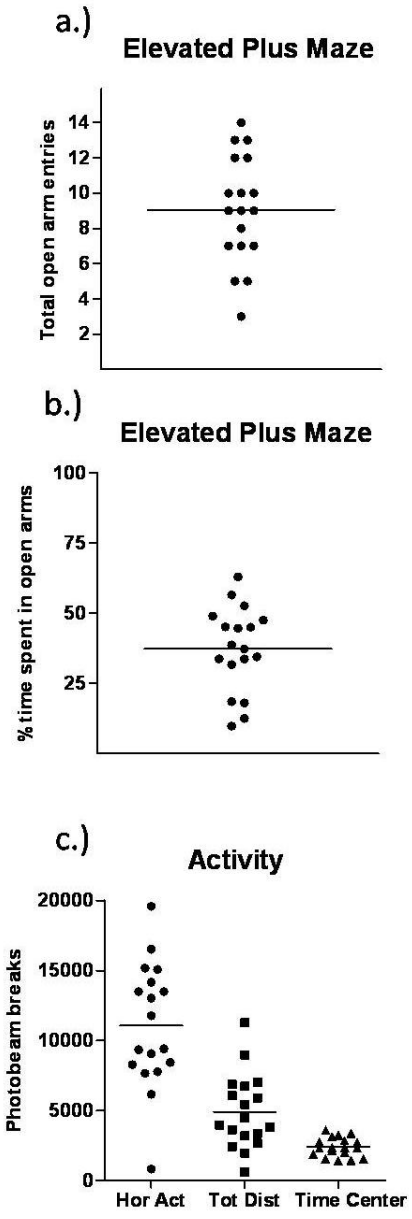


Figure 7: a.) Distribution of scores for total arm entries in elevated plus maze. b.) Distribution of scores for % time spent in open arms of elevated plus maze. c.) Distribution of horizontal activity, total distance, and time spent in the center of the open field scores.

Table 4: Correlation matrix displaying relationships between anxiety measures. While there were significant correlations between measures within the activity and elevated plus maze scores, there were no correlations between tasks (α= .01).

	EPM: % time open arms	EPM: open arm entries	Activity: Time in center	Activity: Horizontal activity	Activity: Distance travelled
EPM: % time open arms	X	***r* = .71**** ***p* <.01**	*r* = .14 *p* = .59	*r* = .05 *p* = .84	*r* = -.04 *p* = .88
EPM: open arm entries	X	X	*r* = -.10 *p* = .70	*r* = .19 *p* = .46	*r* = .16 *p* = .52
Activity: Time in center	X	X	X	*r* = -.22 *p* = .38	*r* = -.19 *p* = .46
Activity: Horizontal activity	X	X	X	X	***r* = .96**** ***p* < .01**
Activity: Distance travelled	X	X	X	X	X

Impulsive Action Assessment Tasks

Differential Rates of Low Responding (DRL)

Performance on all DRL schedules are displayed in Figure 8. For each of the DRL schedules (DRL 5-s, 10-s, and 20-s), total lever presses was negatively correlated with ratio of correct to premature lever presses (*ps* < .01).

DRL Schedule Comparison

Correlations between all DRL measures are displayed in Table 5. Total lever presses at DRL-5s approached a correlation with lever presses at DRL-10s (r = .48, p =.04). DRL-10 total lever presses was near a negative correlation with DRL-20s ratio (amount of reinforced responses divided by amount of premature responses) (r = -.52, p =.03), approached a positive correlation with DRL-30 lever presses (r = .49, p =.04), and achieved a significant negative correlation with DRL-30 ratio (r = -.63, p <.01). DRL-10 ratio approached a positive correlation with DRL-20 ratio (r = .47, p =.04), and DRL-20 ratio was positively correlated with DRL-30 ratio r = .67, p < .01).

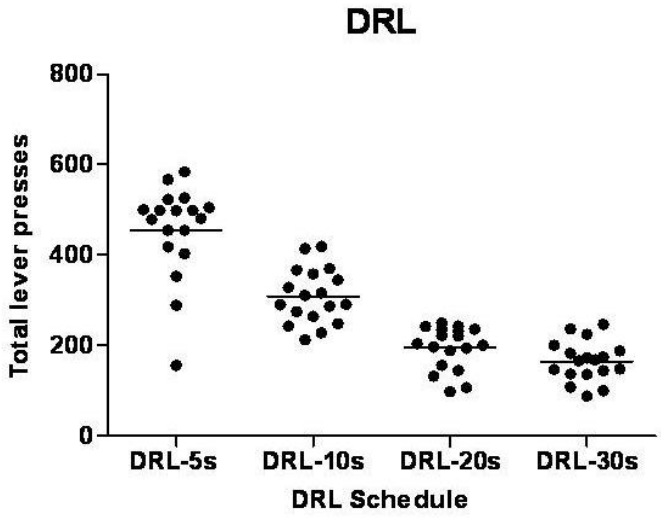
Relationship between Decision-making and Measures from Other Tasks

Correlations between performance in all four decision-making tasks and all other tasks are displayed in Table 6a-b.

Comparisons between Decision-making and Motivation

The only relationship between a decision-making task and a motivation assessment task that approached significance was a negative correlation

a.)



b.)

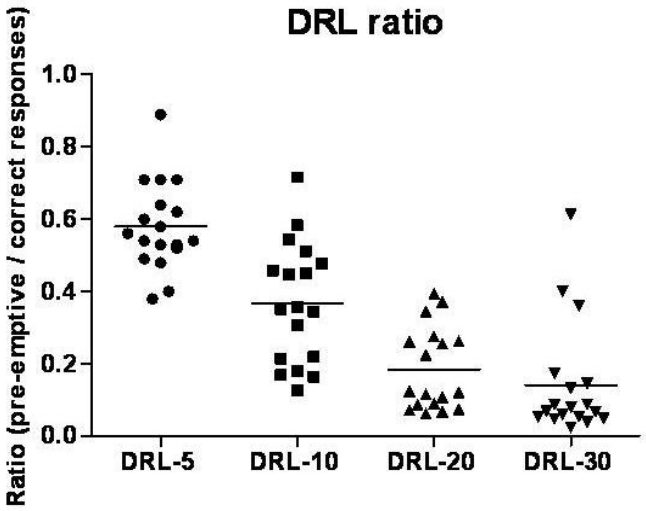


Figure 8: a.) Distribution of total lever presses for each DRL schedule. b.) Distribution of ratio scores for each DRL schedule.

Table 5: Correlation matrix displaying relationships between impulsive action measures. Performance on several different DRL schedules were positively correlated (α= .01).

	DRL-5 **Total LPs**	**DRL-5** Ratio	DRL-10 Total LPs	DRL-10 **Ratio**	DRL-20 **Total LPs**	DRL-20 **Ratio**	**DRL-30 Total LPs**	DRL-30 Ratio
DRL-5 Total LPs	X	***r* = -.92** *p* < .01**	***r* = .48** *p* = .04**	*r* = -.02 *p* = .95	*r* = .27 *p* = .29	*r* = -.23 *p* = .35	*r* = .38 *p* = .12	*r* = -.45 *p* = .06
DRL-5 Ratio	X	X	*r* = -29 *p* = .25	*r* = -.09 *p* = .72	*r* = -.17 *p* = .50	*r* = -.02 *p* = .93	*r* = -.15 *p* = .55	*r* = .24 *p* = .34
DRL-10 Total LPs	X	X	X	***r* = -.70** *p* < .01**	*r* = .22 *p* = .39	***r* = -.52* *p* = .03**	***r* = .49* *p* = .04**	***r* = -.63** *p* < .01**
DRL-10 Ratio	X	X	X	X	*r* = -.28 *p* = .27	***r* = .47* *p* = .05**	*r* = -.04 *p* = .88	*r* = .28 *p* = .26
DRL-20 Total LPs	X	X	X	X	X	***r* = -.64** *p* < .01**	*r* = -.01 *p* = .96	*r* = -.38 *p* = .11
DRL-20 Ratio	X	X	X	X	X	X	*r* = -.36 *p* = .14	***r* = .67** *p* < .01**
DRL-30 Total LPs	X	X	X	X	X	X	X	***r* = -.77** *p* < .01**
DRL-30 Ratio	X	X	X	X	X	X	X	X

Table 6a: Correlation matrix displaying relationships between decision-making, motivation, and pain sensitivity. There were no significant correlations observed (α= .01).

		Risky Decision making	Delay Discounting	Probabilistic Discounting	Effort based discounting
Appetitive motivation	FR1	*r* = .03 *p* = .90	*r* = -.20 *p* = .42	*r* = .04 *p* = .42	*r* = -.08 *p* = .76
	FR3	*r* = .23 *p* = .36	*r* = -.30 *p* = .22	*r* = .16 *p* = .53	*r* = -.15 *p* = .55
	FR10	*r* = .18 *p* = .49	*r* = -.08 *p* = .75	*r* = -.08 *p* = .75	*r* = -.23 *p* = .36
	FR20	*r* = .23 *p* = .37	*r* = -.20 *p* = .41	*r* = .14 *p* = .58	*r* = -.23 *p* = .36
	FR40	*r* = .01 *p* = .97	*r* = -.17 *p* = .51	*r* = .21 *p* = .41	*r* = -.23 *p* = .36
	PR	*r* = -.14 *p* = .57	*r* = -.15 *p* = .54	*r* = .15 *p* = .56	*r* = -.21 *p* = .41
Consummatory motivation	2.5% sucrose	*r* = .21 *p* = .41	*r* = -.25 *p* = .32	*r* = -.38 *p* = .14	*r* = .19 *p* = .44
	5% sucrose	*r* = -.06 *p* = .81	*r* = .07 *p* = .79	*r* = .01 *p* = .96	*r* = .15 *p* = .55
	10% sucrose	*r* = .25 *p* = .33	*r* = -.33 *p* = .18	***r* = -.56*** ***p* = .02**	*r* = -.04 *p* = .88
	20% sucrose	*r* = .33 *p* = .18	*r* = -.12 *p* = .63	*r* = -.32 *p* = .21	*r* = .40 *p* = .10
Pain tolerance	Shock locomotion	*r* = -.23 *p* = .42	*r* = -.19 *p* = .52	*r* = -.08 *p* = .80	*r* = .05 *p* = .88
	Tail Flick	*r* = -.02 *p* = .94	*r* = -.02 *p* = .93	*r* = .35 *p* = .17	*r* = -.22 *p* = .39
	Shock reactivity: motor	*r* = .24 *p* = .38	*r* = -.39 *p* = .14	*r* = -.01 *p* = .98	*r* = -.36 *p* = .17
	Shock Reactivity: vocal	*r* = .15 *p* = .59	*r* = -.34 *p* = .20	*r* = -.05 *p* = .85	*r* = -.35 *p* = .19

Table 6b: Correlation matrix displaying relationships between decision-making, anxiety, and impulsive action. There were significant negative correlations observed between impulsive action ratio and risk decision-making (high risk predicts high impulsive action), and between delay discounting and impulsive action (high impulsive choice predicts high impulsive action) (α= .01).

		Risky Decision making	Delay Discounting	Probabilistic Discounting	Effort based discounting
	Baseline Weight	*r* = .18 *p* = .49	*r* = -.16 *p* = .53	*r* = -.06 *p* = .82	*r* = -.07 *p* = .77
Anxiety	EPM: % time open arms	*r* = -.12 *p* = .62	*r* = -.42 *p* = .09	*r* = .12 *p* = .65	***r* = -.47*** ***p* = .05**
Anxiety	EPM: open arm entries	*r* = -.14 *p* = .59	*r* = -.18 *p* = .47	*r* = .39 *p* = .13	*r* = -.26 *p* = .30
Anxiety	Activity: Time in center	*r* = -.36 *p* = .15	*r* = -.18 *p* = .48	*r* = .08 *p* = .77	*r* = -.27 *p* = .28
Anxiety	Activity: Horizontal activity	*r* = .23 *p* = .35	*r* = .13 *p* = .63	*r* = -.27 *p* = .29	*r* = .46 *p* = .06
Anxiety	Activity: Distance travelled	*r* = .29 *p* = .24	*r* = .12 *p* = .63	*r* = -.30 *p* = .24	*r* = .40 *p* = .10
Impulsive action	DRL-5 Total LPs	*r* = -.09 *p* = .72	***r* = -.52*** ***p* = .03**	*r* = .04 *p* = .87	*r* = -.30 *p* = .22
Impulsive action	DRL-5 Ratio	*r* = .27 *p* = .28	*r* = .40 *p* = .10	*r* = -.12 *p* = .65	*r* = .30 *p* = .23
Impulsive action	DRL-10 Total LPs	*r* = .26 *p* = .30	*r* = -.39 *p* = .11	*r* = .14 *p* = .59	*r* = -.17 *p* = .49
Impulsive action	DRL-10 Ratio	*r* = -.23 *p* = .35	*r* = .13 *p* = .60	*r* = -.15 *p* = .56	*r* = .12 *p* = .63
Impulsive action	DRL-20 Total LPs	*r* = .37 *p* = .13	***r* = -.58**** ***p* = .01**	*r* = -.11 *p* = .67	*r* = -.11 *p* = .68
Impulsive action	DRL-20 Ratio	***r* = -.65**** ***p* < .01**	*r* = .31 *p* = .22	*r* = -.19 *p* = .46	*r* = .02 *p* = .92
Impulsive action	DRL-30 Total LPs	*r* = .23 *p* = .36	***r* = -.48*** ***p* = .04**	*r* = -.18 *p* = .50	***r* = -.49*** ***p* = .04**
Impulsive action	DRL-30 Ratio	*r* = -.45 *p* = .06	***r* = .53*** ***p* = .02**	*r* = -.16 *p* = .55	*r* = .37 *p* = .13

between probabilistic discounting and consumption of 10% sucrose solution (r = -.56, p = .02), meaning that rats with a preference for the large, probabilistic reward were less likely to consume large amounts of sucrose at this concentration. Multiple regression analyses were performed analyzing the ability of all sucrose consumption variables to predict decision-making: there were no significant relationships between either delay discounting, effort-based discounting, or risky decision-making with a composite of sucrose consumption performance variables (ps > .31), and a near significant predictive relationship between sucrose consumption and probabilistic discounting ($F_{(4,17)}$ = 2.72, p = .08). Similar analyses between the decision-making tasks and a composite of all instrumental responding measures revealed a near-significant relationship between instrumental responding (appetitive motivation) and risky decision-making ($F_{(6,17)}$ =2.71, p = .07). Finally, each decision-making task was regressed upon composites of all motivation variables. There was a near significant relationship between composite motivation and risky decision-making ($F_{(10,17)}$ = 3.62, p = .05), although the predictive weights of the variables were inconsistent and difficult to interpret (for example, FR20 was positively associated with risk, while FR40 was negatively associated). There were no predictive relationships between motivation and the other decision-making tasks (ps > .31).

Comparisons between Decision-making and Pain Tolerance

There were no correlations between any of the decision-making tasks and any of the pain assessment measures (ps > .17). The decision-making tasks

were each regressed upon a composite of all pain assessment measures, and none of these predictive relationships were significant (*ps* > .19).

Comparisons between Decision-making and Anxiety

Only one correlation approached significance between a decision-making task and any of the measures of anxiety: there was a negative relationship between effort-based discounting and percent of time spent in the open arms of the EPM (r = -.47, p = .05). This indicated that rats with a preference for the large, effort-requiring reward tended to spend a smaller percentage of time in the open arms (an indication of greater anxiety). Each decision-making measure was regressed upon a composite of all anxiety measures. None of these relationships were significant, although the predictive relationship between anxiety and effort-based discounting approached significance ($F_{(5,17)}$ = 2.89, p = .06).

Comparisons between Decision-making and Impulsive Action

Several of the impulsive action assessment protocols were correlated with measures of decision-making. There was a significant negative correlation between large reward choice in the delay discounting task and total responses on the DRL-20 (r = -.58, p = .01), indicating that subjects that discounted the large reward to a greater degree also performed more inaccurate responses on the DRL (i.e., greater impulsive action was correlated with greater impulsive choice). Additionally, large reward preference on the risky decision-making task was negatively correlated with DRL-20 response ratio (r = -.65, p <.01), indicating that rats that preferred the risky option also performed a higher ratio of premature, impulsive responses on the DRL. There were also several near-

significant correlations between delay discounting and DRL measures (DRL-5 total lever presses, r = -.52, p = .03; DRL-30 total lever presses, r = -.48, p = .04; DRL-30 ratio, r = .53, p = .02), providing further evidence that impulsive choice is related to impulsive action. Finally, effort-based discounting approached a significant negative correlation with DRL-30 total lever presses (r = -.49, p = .04).

Each decision making task was then regressed upon composites of the different DRL test schedules. No combination of DRL variables was significantly predictive of probabilistic discounting or effort-based discounting (ps > .30). A combination of all total lever press scores on all DRL schedules was a near significant predictor of risky decision-making ($F_{(4,17)}$ = 3.30, p = .05), and the combination of all lever press scores was a significant predictor of delay discounting ($F_{(4,17)}$ = 4.96, p = .01) such that high impulsive action predicted high impulsive choice.

Comparisons between Decision-making and Multiple Measures

Each decision-making task was regressed upon combinations of different categories of variables (for example, motivation and pain sensitivity scores were combined and used to predict decision-making. None of these combinations significantly predicted any of the four decision-making tasks (ps > .09), although a combination of motivation and pain measures was near-significantly predictive of probabilistic discounting (p = .03).

Experiment 2 Results

Amphetamine

There was a main effect of drug dose ($F_{(3,33)}$ = 3.87, p <.05; Figure 9) such that rats became more risk-averse with increasing doses of amphetamine (although the punishment probability x drug interaction did not quite reach significance [$F_{(12,132)}$ = 1.80, p =.055]). Individual pair-wise comparisons between saline and amphetamine conditions showed that the 1.5 mg/kg dose caused a significant decrease in preference for the large reward (p < .05). In addition to its effects on reward choice, amphetamine also increased the number of omitted trials ($F_{(3,33)}$ = 3.92, p < .05), with omissions increasing as a function of dose (% completed choice trials: saline=98.66, .33 mg/kg=94.00, 1.0 mg/kg=94.34, 1.5 mg/kg=84.00). However, the effects of amphetamine on omissions appeared to be separate from its effects on reward choice, as there were no correlations between these two variables (rs <.35, *ns*). There was also no difference in shock reactivity (locomotion during the 1 s shock presentations) across drug doses ($F_{(3,21)}$ = .12, *n.s.*).

α-Flupenthixol

There was no effect of flupenthixol administration on choice behavior $F_{(3,33)}$ = 1.44, p =.25; Figure 10), nor was there an interaction between punishment probability and drug ($F_{(12,132)}$ = 1.02, p = .44). There was also no effect on trials omitted ($F_{(3,33)}$ = 2.26, p = .10).

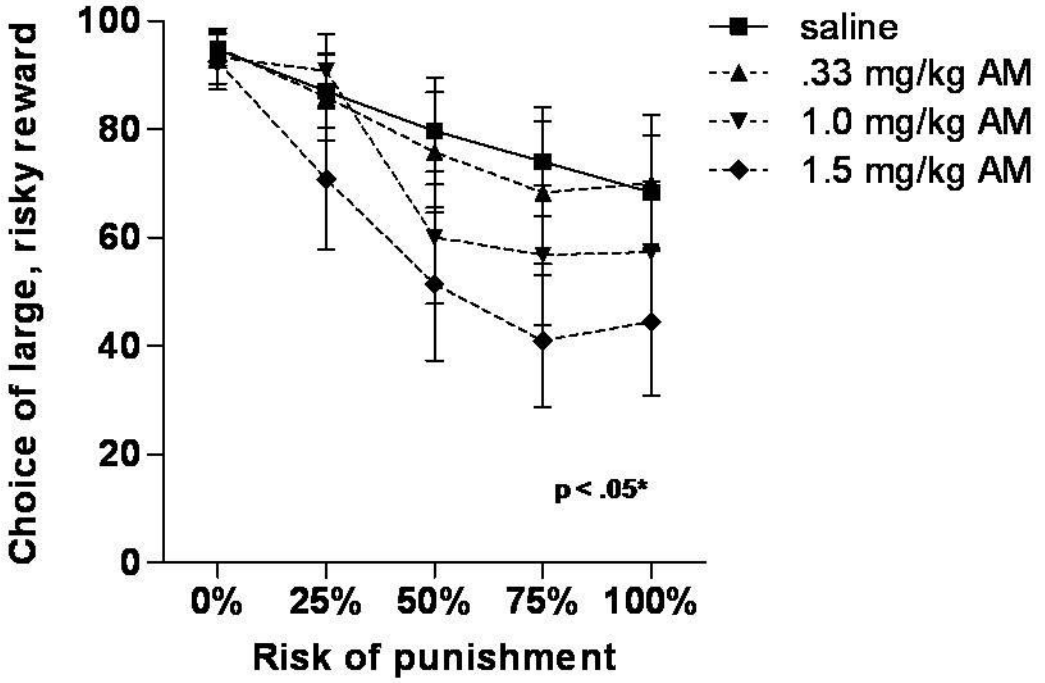


Figure 9: Rats were tested under the influence of systemic .33, 1.0, and 1.5 mg/kg doses of amphetamine. Amphetamine decreased preference for the large reward in a dose-dependent fashion, with the 1.5 mg/kg dose differing significantly from saline conditions ($p < .05$).

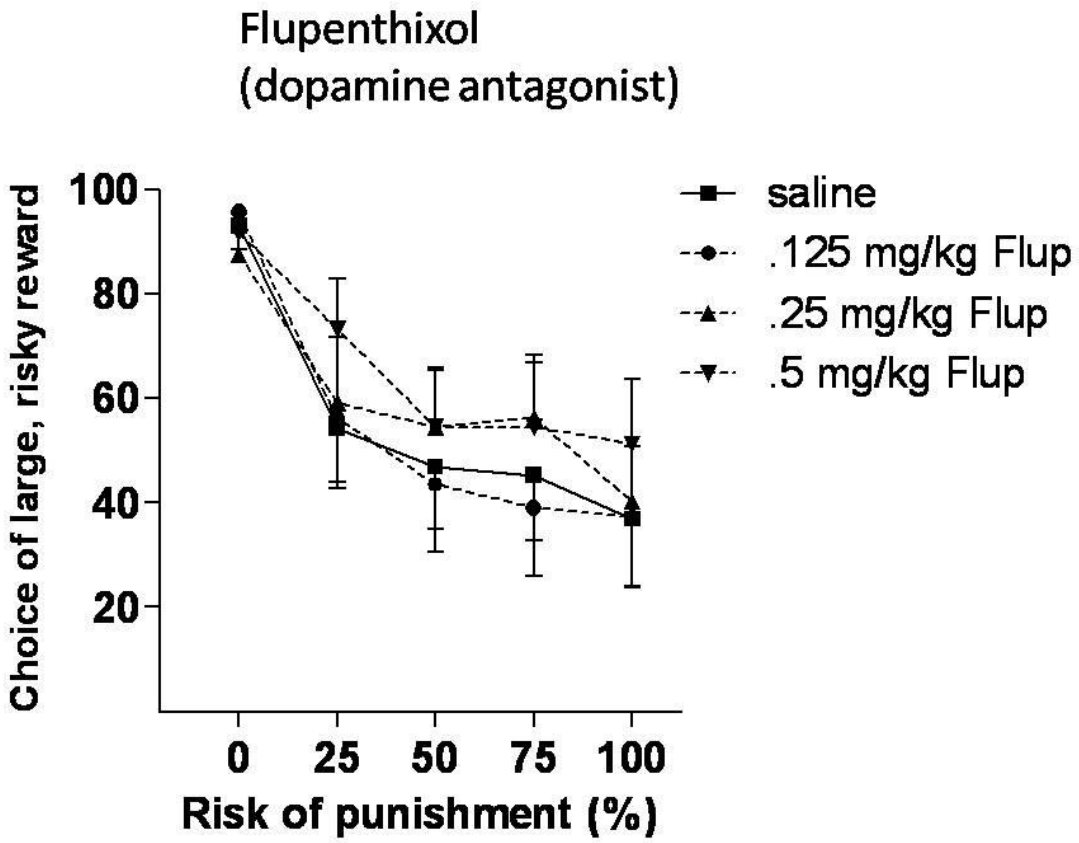


Figure 10: Rats were tested under the influence of systemic .125, .25, and .5 mg/kg doses of the dopaminergic antagonist α-flupenthixol. Flupenthixol administration did not affect risky choice.

D1 Dopamine Receptor Manipulation

SKF81297(D1 Agonist)

There was no effect of acute SKF81297 (D1 agonist) treatment on reward preference ($F_{(3,33)}$ = 1.73, p =.18; Figure 11a), nor was there an interaction between drug dose and punishment probability ($F_{(12,132)}$ = .83, p = .62). There was no effect of drug dose on trials omitted (although it did approach significance: $F_{(3,33)}$ = 2.44, p = .08). There was also a non-significant trend toward a drug-induced difference in locomotion ($F_{(3,33)}$ = 2.85, p = .053) such that the highest dose of SKF81297 (1.0 mg/kg) decreased baseline locomotion during the task compared to saline and the other two doses of SKF81297. There was no difference in locomotion during an average of all one second shock periods ($F_{(3,21)}$ = .44, p = .73).

SCH23390 (D1 Antagonist)

There was no effect of acute SCH23390 (D1 antagonist) on reward choice ($F_{(3,33)}$ = .03, p = .99; Figure 11b). There was no interaction between drug dose and punishment probability ($F_{(12,132)}$ = .92, p = .53). There was a significant effect of SCH23390 treatment on trials omitted such that the highest dose resulted in the highest percentage of trail omitted ($F_{(3,33)}$ = 5.27, p <.05; % completed choice trials: saline=99%, low =99.17%, mid = 99.33, high = 85%). There was also a significant dose-dependent difference in baseline locomotion during the task ($F_{(3,33)}$ = 3.63, p < .05) such that acute SCH23390 reduced locomotion compared

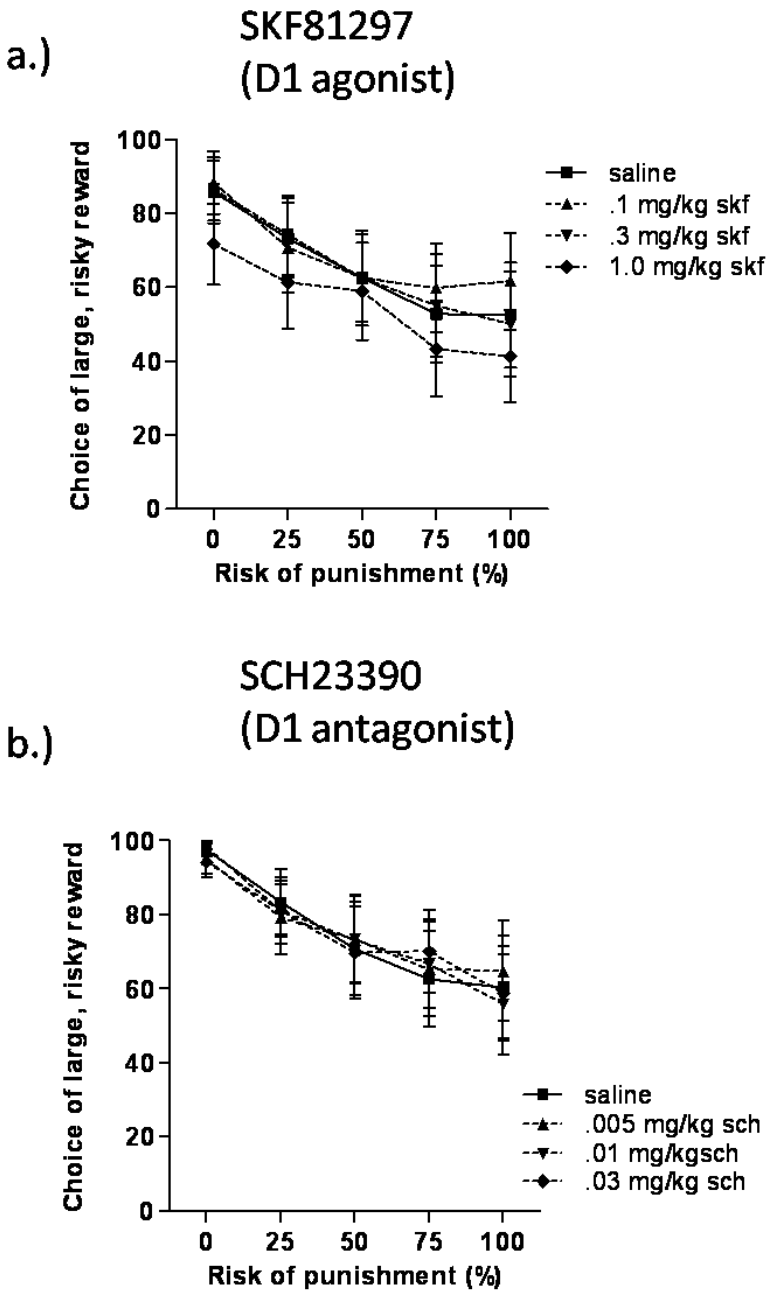


Figure 11: Rats were tested using drugs that target D1 dopamine receptors. a.) Rats were administered .1, .3, and 1.0 mg/kg skf81297. No effects on risky choice were observed. b.) Rats were tested under the influence of .005, .01, and .03 mg/kg SCH23390. Again, no effects were observed.

to saline. Again, there was no effect of drug treatment on locomotion during the shock periods ($F_{(3,27)}$ = 1.63, p = .21).

D2 Dopamine Receptor Manipulation

Bromocriptine (D2 Agonist)

Bromocriptine (D2 agonist) administration produced a dose-dependent decrease in preference for the risky reward ($F_{(3,33)}$ = 3.37, p = .03; Figure 12a). This increase in risk-aversion qualitatively resembled the effects of amphetamine, with the highest dose inducing the largest shift toward risk averse choice. There was also an interaction between drug dose and punishment probability ($F_{(12,132)}$ = 2.07, p = .02) such that subjects given bromocriptine demonstrated a more substantial shift away from the large, risky reward as the risk of punishment increased than subjects administered saline. There was no effect of bromocriptine vs. saline on baseline locomotion ($F_{(3,33)}$ = 2.09, p = .12), nor was there an effect on trials omitted ($F_{(3,33)}$ = 2.35, p = .09). Additionally, there was no effect of treatment on locomotion during the shock presentations ($F_{(3,33)}$ =.71, p = .56).

Eticlopride (D2 Antagonist)

Eticlopride (D2 antagonist) did not affect risky choice behavior ($F_{(3,33)}$ = .86, p = .47; Figure 12b); there was also no interaction between dose and punishment probability ($F_{(12, 312)}$ = .71, p = .74). There was no effect of eticlopride on trials omitted ($F_{(3,33)}$ = .59, p = .63), nor any effect on baseline locomotion ($F_{(3,33)}$ = 1.70, p = .19) or shock locomotion ($F_{(3,21)}$ = 1.70, p = .19).

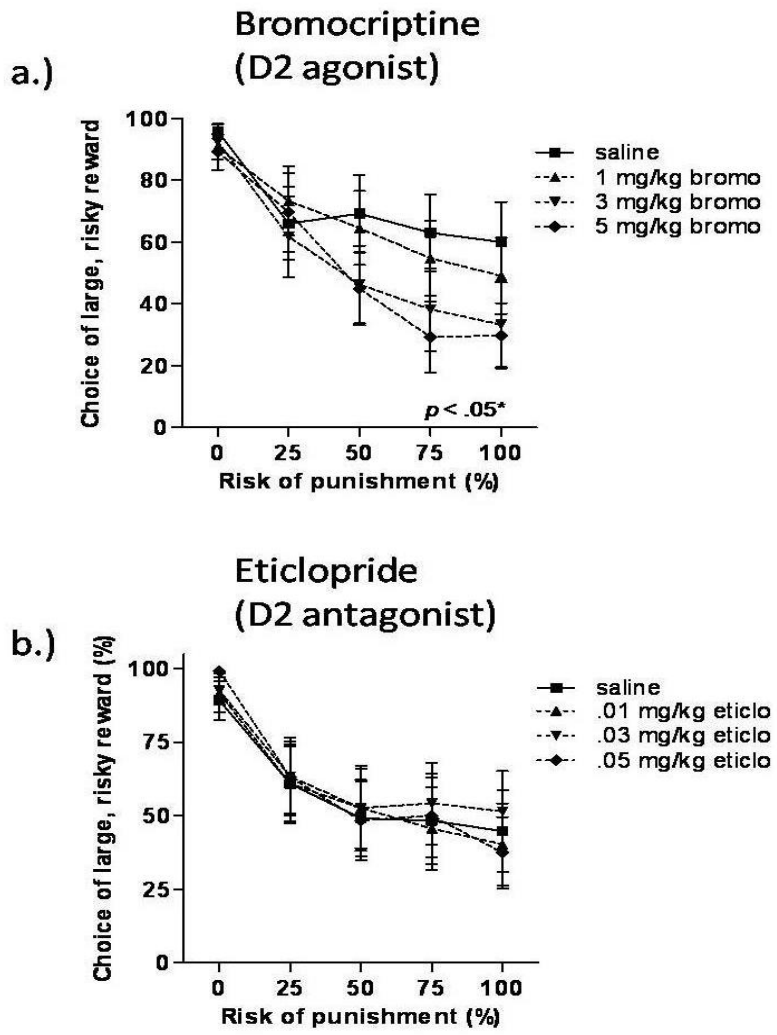


Figure 12: Rats were tested using drugs that target D2 dopamine receptors. a.) Rats were administered 1, 3, and 5.0 mg/kg bromocriptine. Bromocriptine induced a dose-dependent attenuation of risky choice ($p < .05$). b.) Rats were tested under the influence of .01, .03, and .05 mg/kg eticlopride. No effects on risky decision-making were observed.

Combined Amphetamine and Antagonist Administration

Amphetamine and SCH 23390 Coadministration

In order to determine whether amphetamine's effects were acting through D1 or D2 receptors, amphetamine was co-administered with either a D1 or D2 antagonist. Following combined amphetamine (5mg/kg) and SCH 23390 (D1 antagonist; .03 mg/kg) administration, there was a significant effect of drug ($F_{(2,22)}$ = 3.65, p < .05; Figure 13) such that both amphetamine and amphetamine coadministered with SCH23390 decreased preference for the risky reward relative to saline, There was also a significant drug X punishment probability interaction $F_{(4,44)}$ = 3.31, p < .01). Individual comparisons revealed that there was a significant difference between saline treatment and amphetamine + SCH co-administration ($F_{(2,22)}$ = 5.13, p < .05) such that the combined drug treatment attenuated risky choice, and a near-significant difference between amphetamine treatment and saline treatment ($F_{(2,22)}$ = 4.40 p = .06). There was no difference between amphetamine treatment and amphetamine and SCH co-administration (p = .60), indicating that the effects of amphetamine on risky choice persist even in the absence of D1 dopamine receptor activation. There was no difference in trials omitted between groups ($F_{(2,22)}$ = .71 p = .51). There was an effect of drug treatment on baseline locomotion ($F_{(2,22)}$ = 6.88 p < .01) such that both amphetamine and amphetamine + SCH co-administration caused more locomotor activity than saline treatment.

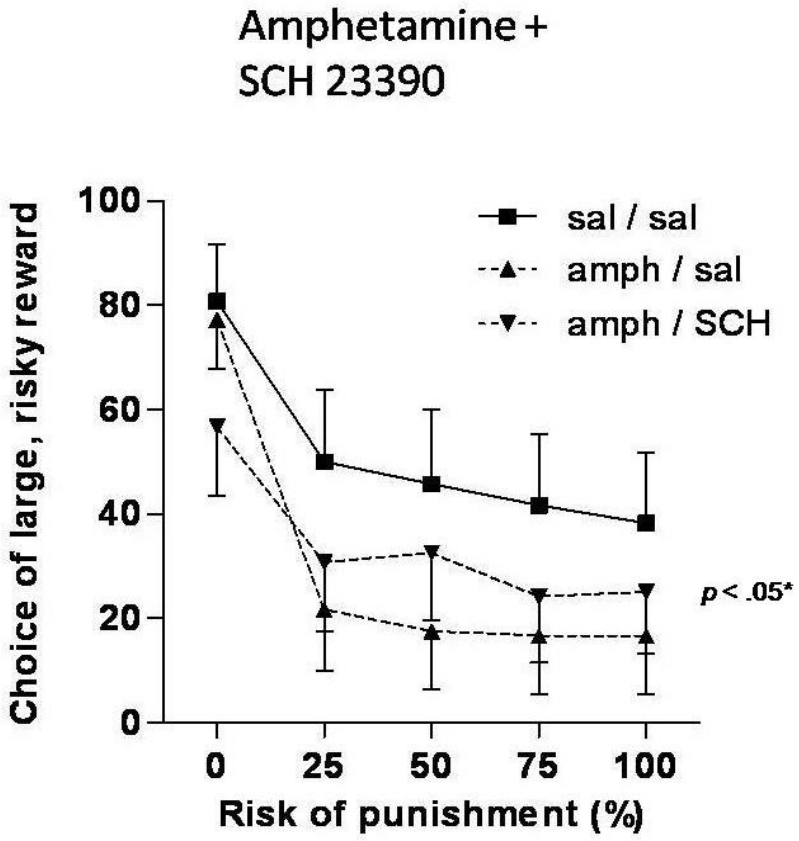


Figure 13: Rats were tested under the influence of either saline, amphetamine alone (5 mg/kg), or amphetamine (5 mg/kg) coadministered with the D1 antagonist SCH 23390 (.03 mg/kg). Amphetamine administered with SCH 23390 attenuated risky choice in the same fashion as amphetamine (*p* < .05), indicating that D1 receptor activation is not necessary for amphetamine's effects on risky choice.

Amphetamine and Eticlopride Coadministration

There was an effect of drug schedule on reward preference ($F_{(2,22)}$ = 4.22, p < .05; Figure 14) and an interaction between drug and block ($F_{(8,88)}$ = 3.94, p < .01). This interaction indicated that amphetamine decreased risky choice behavior in comparison to saline treatment, but amphetamine co-administered with eticlopride did not affect choice behavior (i.e., the D2 antagonist blocked the effects of amphetamine). Further statistical evidence for this effect was obtained by performing ANOVA comparing individual treatments; rats given saline and amphetamine showed significantly more risk aversion than rats given only saline ($F_{(1,11)}$ = 5.89, p <.05), while, importantly, there was no significant difference between rats given eticlopride + amphetamine and those given saline only ($F_{(1,11)}$ = 1.01, p =.34). Additionally, there was a near significant difference between performance under acute amphetamine and a combination of amphetamine and eticlopride ($F_{(1,11)}$ = 4.16, p =.066) such that rats given amphetamine demonstrated a larger shift toward risk-averse choice than rats given the combination of drugs. There was no effect of drug administration on trials omitted ($F_{(2,22)}$ =.60, p =.56). Interestingly, there was an effect of drug on overall locomotion during testing ($F_{(2,22)}$ =19.19, p <.01) such that administration of amphetamine or amphetamine with eticlopride both increased locomotion relative to saline (i.e. –eticlopride did not block the locomotor stimulant effect of ampmethamine). There was no effect of drug treatment on average locomotion during the shock periods ($F_{(2,6)}$ =.63, p =.55).

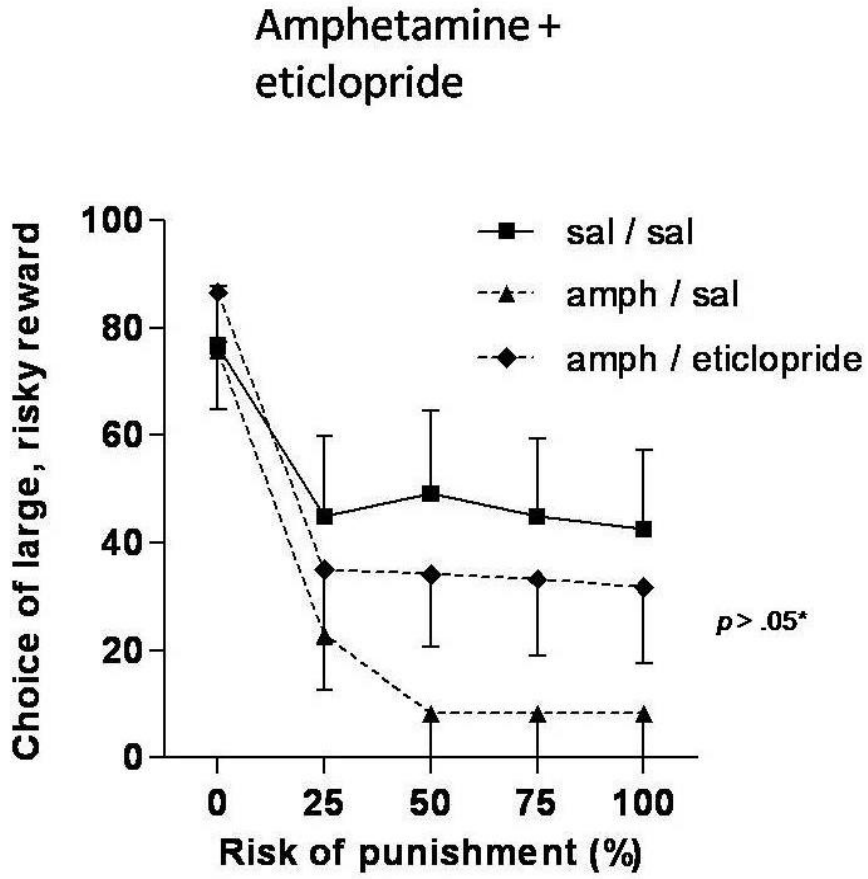


Figure 14: Rats were tested under the influence of either saline, amphetamine alone (5 mg/kg), or amphetamine (5 mg/kg) coadministered with the D2 antagonist eticlopride (.05 mg/kg). Amphetamine administered independently attenuated risky choice (p < .05), but amphetamine coadministered with eticlopride did not affect risky decision-making. This indicated that D2 receptor activation is necessary for amphetamine's effects on risky choice.

Experiment 3 Results

Because the D2 rather than D1 dopamine receptor had been demonstrated to mediate risky decision-making behavior in Experiment 2, D2 receptor cRNA was chosen for this experiment. Figure 15 shows levels of D2 dopamine cRNA hybridization in prefrontal cortical-striatal circuitry from behaviorally characterized animals (orbitofrontal cortex [OFC], medial prefrontal cortex [mPFC], dorsal striatum [DS], and nucleus accumbens [NAcc]). Sample hemisections, as well as the regions analyzed, are displayed as Figure 16.

The Relationship between D2 Dopamine Receptor cRNA Hybridization and Risky Decision-making Behavior

There were no significant linear correlations between risky decision-making performance and D2 cRNA hybridization in any of the brain regions analyzed (*ps* > .17). However, regionally-specific monoamine transmission often mediates behavior in a non-linear fashion, manifested as a U- or inverted U-shaped curve (Cai & Arnsten 1997; Robbins 2005), which would be undetectable utilizing linear correlations. To address this possibility, nonlinear curve estimation analyses were also performed, specifically to determine if the relationship between data sets was best reflected by a quadratic function. Additionally, to further confirm the relationship between risk and D2 cRNA hybridization, rats were split evenly into three groups based on task performance: risk-averse, moderate risk, or risk-taking (Figure 17). Region-specific D2 cRNA hybridization was then compared between these groups using one way ANOVAs.

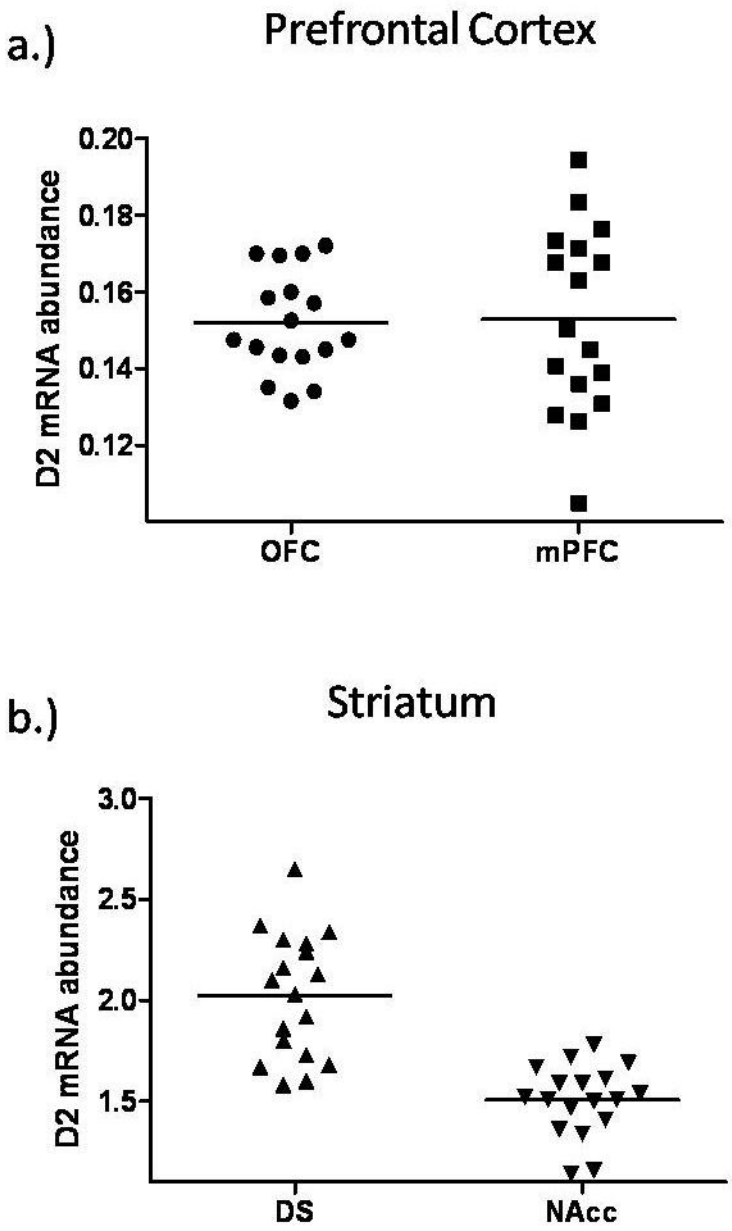


Figure 15: a.) Distribution of D2 mRNA hybridization signals in prefrontal cortical areas. OFC = orbitofrontal cortex, mPFC = medial prefrontal cortex. b.) Distribution of D2 mRNA hybridization signals in striatum. DS = dorsal striatum, NAcc = nucleus accumbens.

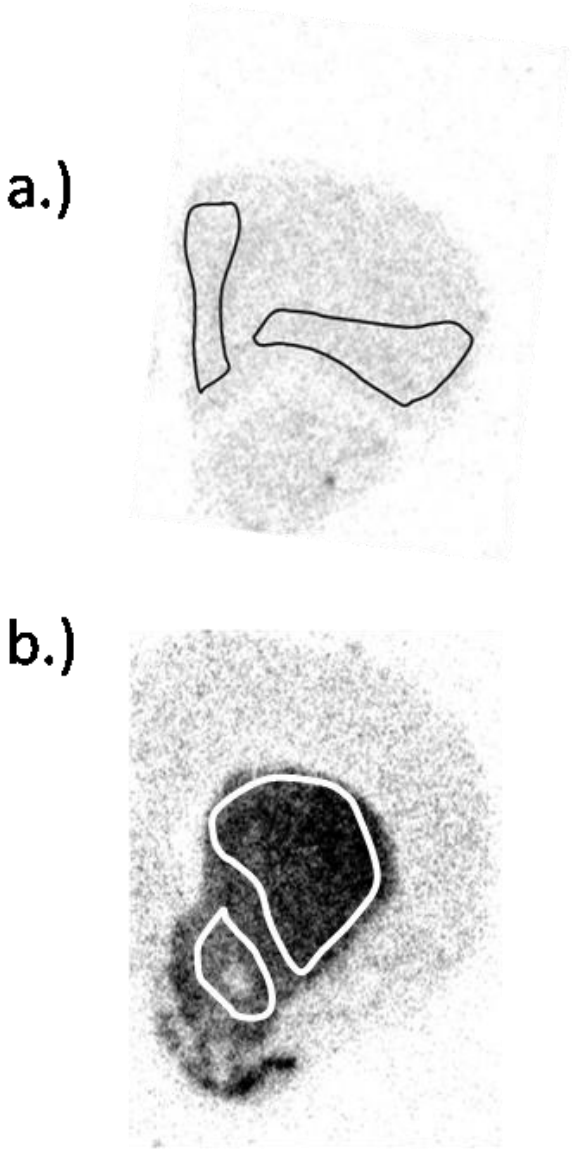


Figure 16: a.) Sample hybridized hemisection containing mPFC (prefrontal region outlined along the midline) and OFC (located dorsal to the olfactory bulb and lateral to mPFC) regions. b.) Sample hybridized hemisection containing DS (ventral to corpus callosum) and NAcc (surrounding the anterior portion of anterior commisure) regions.

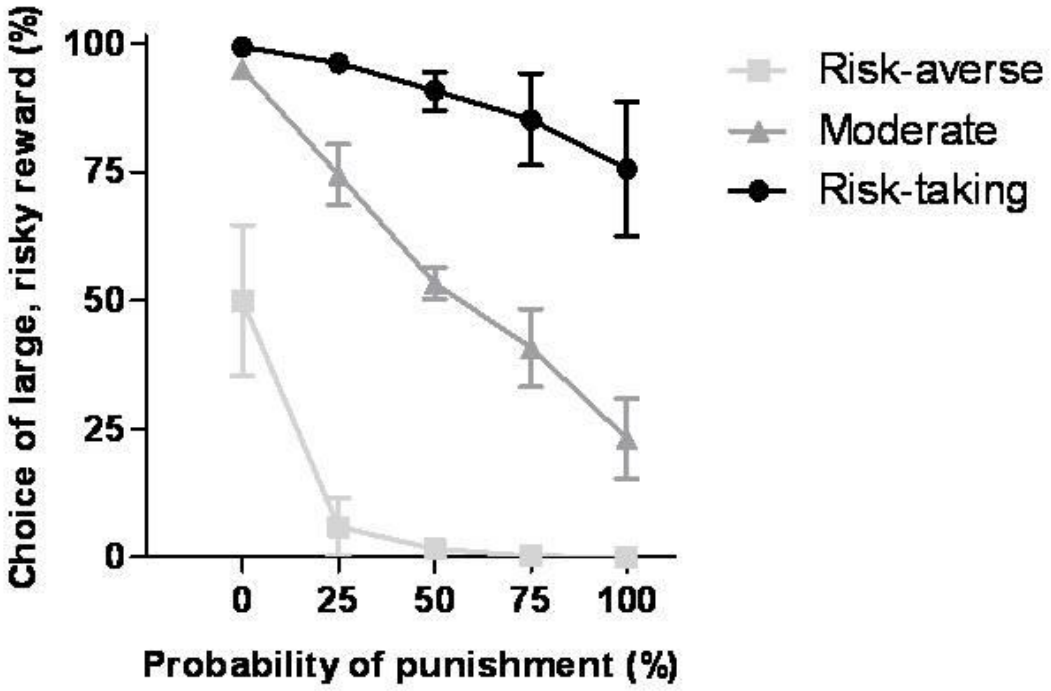


Figure 17: Rats were divided into three evenly sized groups based on risky decision-making performance: risk-averse, moderate, and risk-taking.

Prefrontal Cortex

There was a significant quadratic relationship between OFC hybridization and risk that resembled a U curve ($F_{(2,14)} = 4.46$, $p < .05$; Figure 18a). There was also an effect of risk level on D2 cRNA hybridization in OFC ($F_{(2,14)} = 7.60$, $p < .05$) such that the risk-averse and risk-taking groups both displayed a greater hybridization signal than the moderate risk group (Figure 18b). These relationships were confirmed statistically by LSD post hoc analyses (*ps* < .01). Interestingly, a contrasting nonlinear function was found in mPFC, with the relationship between mPFC and risk resembling an inverted U curve ($F_{(2,14)} = 5.60$, $p < .05$; Figure 18c). There was also an effect of risk level on hybridization in mPFC ($F_{(2,14)} = 4.32$, $p < .05$; such that the moderate risk group demonstrated a higher hybridization signal than the risk-averse or risk-taking groups (Figure 18d). LSD post hoc analyses revealed that the moderate risk group had higher levels of cRNA hybridization in mPFC than the risk-taking group ($p < .05$), and there were no other differences between groups (*ps* > .18).

Striatum

There was a significant nonlinear relationship between D2 hybridization in DS and risk that resembled a modified U curve ($F_{(2,15)} = 7.26$, $p < .01$; Figure 19a). An ANOVA revealed a significant effect such that the risk averse group exhibited a higher hybridization signal than the moderate risk and risk-taking groups, and the risk-taking group showed a higher signal than the moderate risk group ($F_{(2,15)} = 6.52$, $p < .01$; Figure 19b). LSD post hoc comparisons confirmed that hybridization was significantly greater in the risk-averse group than in

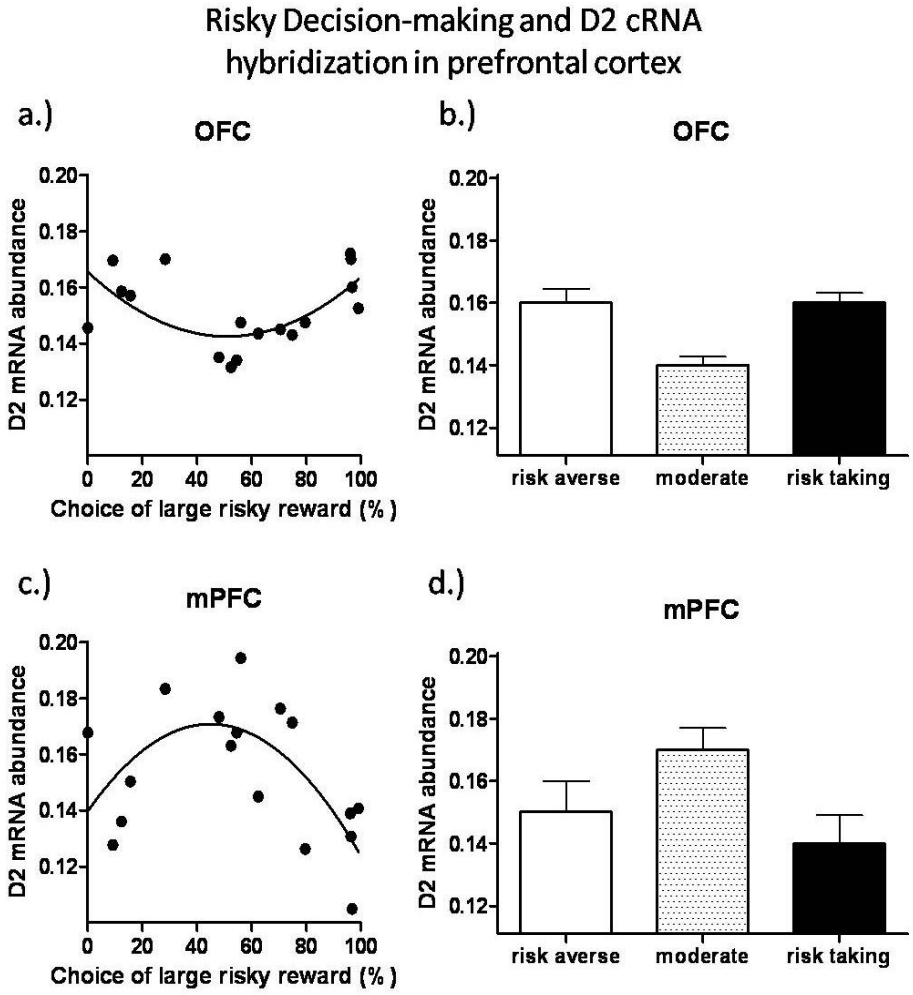


Figure 18: a.) There was a quadratic relationship between OFC D2 mRNA and risky decision-making ($p < .05$). b.) D2 mRNA hybridization in OFC was greater in the risk averse and risk taking groups than in the moderate risk group ($p < .05$). c.) There was an inverted U-shaped quadratic relationship between D2 mRNA in mPFC and risky choice ($p < .05$). d.) The moderate risk group displayed greater D2 mRNA hybridization in mPFC than the risk-taking or risk-averse groups ($p < .05$).

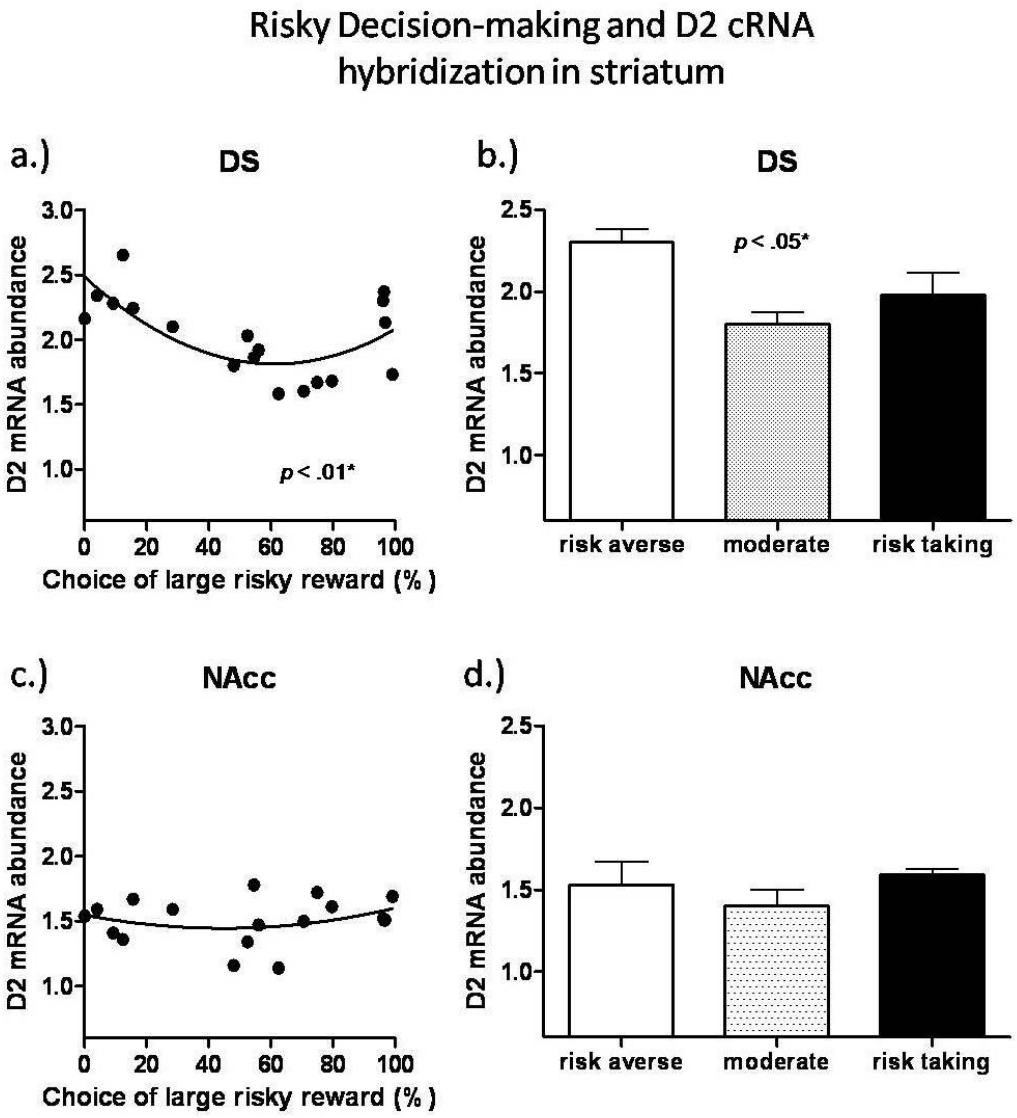


Figure 19: a.) There was a quadratic relationship between DS D2 mRNA and risky decision-making ($p < .01$). b.) D2 mRNA hybridization in DS was greater in the risk averse than both the risk taking and moderate risk groups ($p < .05$). c.) There was no relationship between D2 mRNA in NAcc and risky choice. d.) There was no difference in NAcc D2 hybridization between groups.

both the moderate risk and the risk-taking groups ($p < .01$), while a comparison showed that the moderate risk and risk-taking groups were not significantly different ($ps = .21$). There was neither a significant quadratic relationship between NAcc cRNA hybridization and risky decision-making ($p = .50$), nor any difference between risk levels in D2 cRNA hybridization in NAcc ($F_{(2,15)} = 2.19$, $p = .15$) (Figure 19 c-d).

The Relationship between D2 Dopamine Receptor cRNA Hybridization and Other Cost-benefit Decision-making Tasks

Probabilistic Discounting and Prefrontal Cortex

Performance on the probabilistic discounting task approached a negative linear correlation with cRNA hybridization in OFC ($r = 1.52$, $p = .03$; Figure 20a), such that preference for the large, probabilistic risky reward was associated with a lower abundance of D2 mRNA. This relationship was analyzed further by dividing subjects into three even groups based on probabilistic discounting performance (high prob, mid prob, low prob, with the high prob group corresponding with the highest level of probabilistic risk), then performing an ANOVA comparing D2 hybridization between probability groups, with the low prob group displaying a higher level of D2 cRNA hybridization in OFC than both the mid prob and high prob groups ($F_{(2,15)}=8.74$, $p < .01$, Figure 20b). These group differences were confirmed with LSD post hoc analyses ($ps < .01$).

Additionally, D2 cRNA hybridization in mPFC reached a near-significant positive correlation with probabilistic discounting ($r = .48$, $p = .053$; Figure 20c) indicating the trend that rats with preference for the large, probabilistic reward

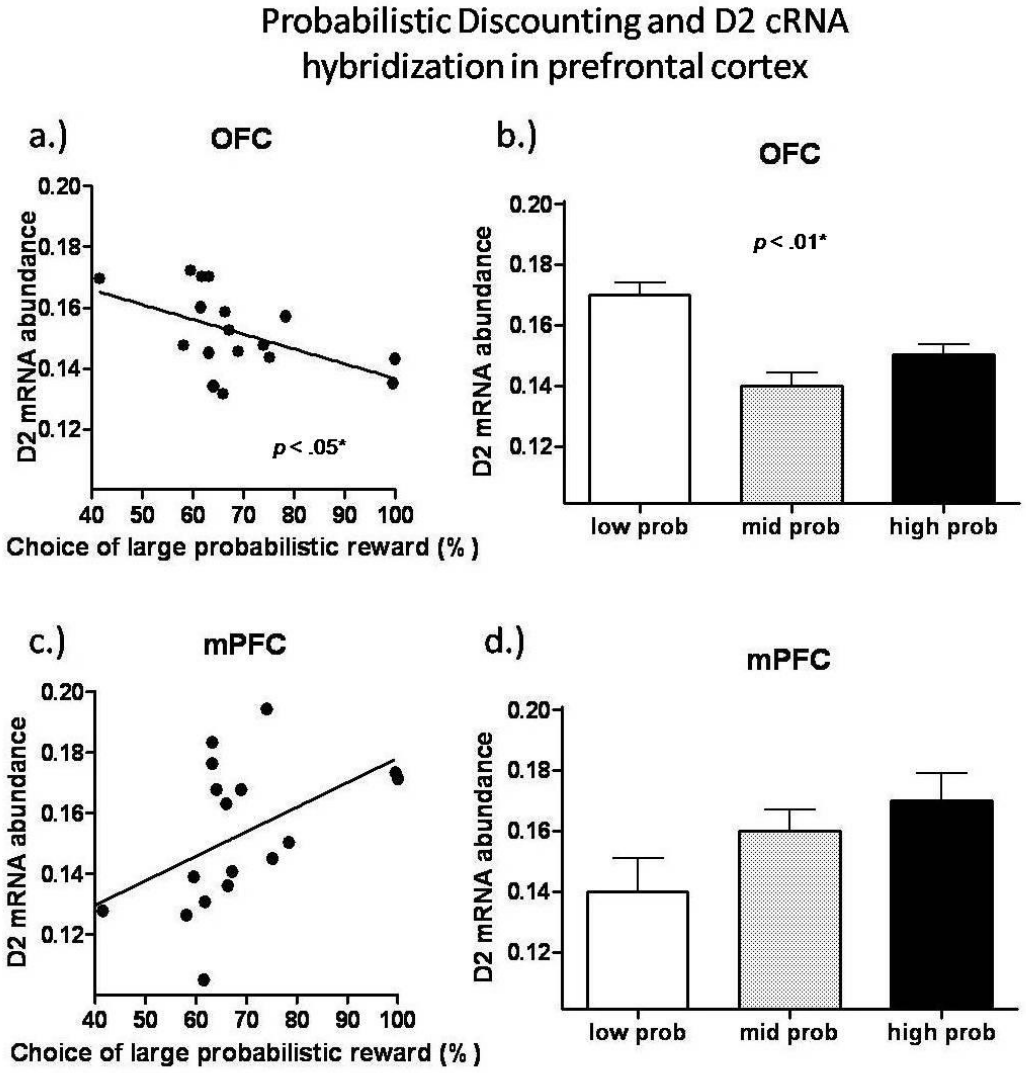


Figure 20: a.) There was a linear negative correlation between OFC D2 mRNA and probabilistic discounting ($p < .05$). b.) D2 mRNA hybridization in OFC was greater in the risk averse than both the risk taking and moderate risk groups ($p < .01$). c.) There was no significant relationship between D2 mRNA in mPFC and probabilistic discounting. d.) There was no difference in mPFC D2 hybridization between groups.

were more likely to display higher levels of hybridization in mPFC. An ANOVA comparing D2 cRNA hybridization between groups based on probabilistic discounting performance in mPFC was also near significant ($F_{(2,15)}$ = 3.36, p = .06; Figure 20d).

Probabilistic Discounting and Striatum

There were no significant linear or nonlinear correlations or ANOVAs associating probabilistic discounting with D2 hybridization in either DS or NAcc (*ps* > .17). There were also no effects of group on hybridization in either region (*ps* > .54; Figure 21).

Delay Discounting and Prefrontal Cortex

Delay discounting performance was not significantly linearly correlated with D2 cRNA hybridization in either OFC or mPFC (*ps* > .07). There was no significant non-linear relationship between OFC and choice of the delayed reward (p = .19), nor was there any no effect of group on cRNA hybridization in OFC, although it approached significance (p = .06; Figure 22a-b). Non-linear curve estimation analysis revealed a significant quadratic relationship between D2 cRNA hybridization in mPFC and delay discounting manifested as a U curve ($F_{(2,14)}$ = 10.78, p < .01; Figure 22c). Rats were then broken up into three groups based on delay discounting performance (high impulsivity, mid impulsivity, and low impulsivity, with impulsivity inversely related to large reward preference), and D2 expression was compared between these groups. Within mPFC, both the high impulsivity and low impulsivity groups displayed higher hybridization than the mid impulsivity group ($F_{(2,15)}$ = 3.80, p < .05; Figure 22d). LSD post hoc tests

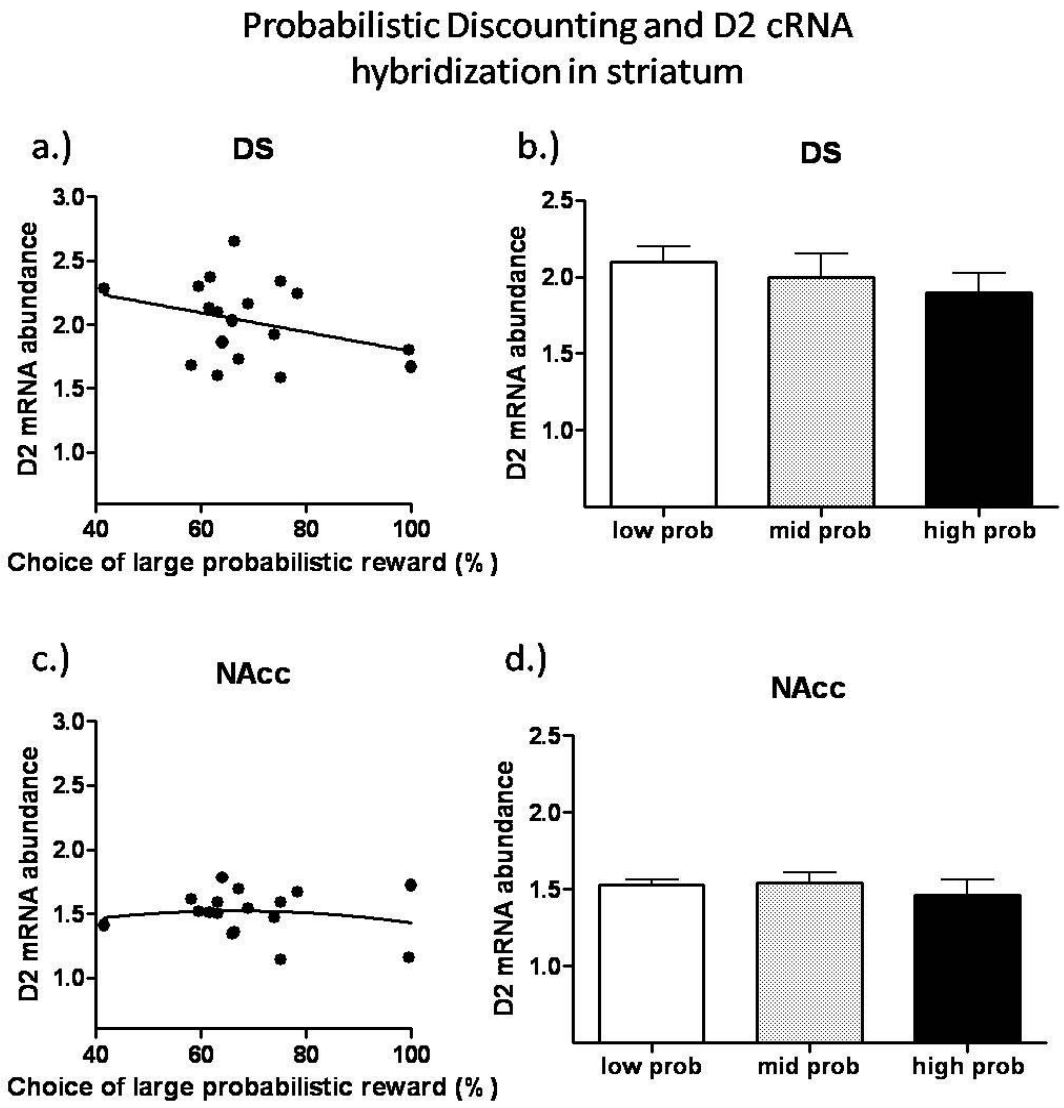


Figure 21: a.) There was no relationship between DS D2 mRNA hybridization and probabilistic discounting. b.) There was no difference in D2 mRNA hybridization in DS between groups. c.) There was no significant relationship between D2 mRNA in NAcc and probabilistic discounting. d.) There was no difference in NAcc D2 hybridization between groups.

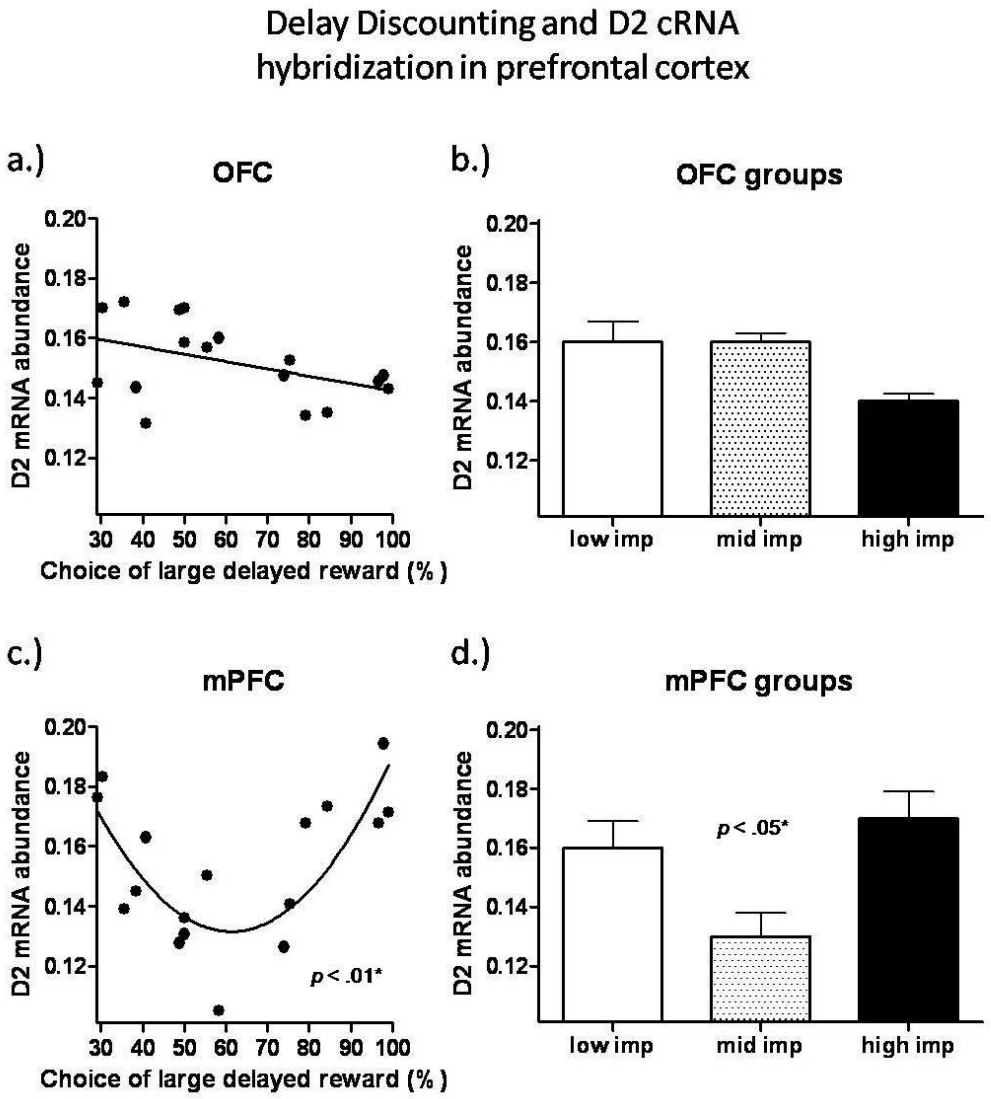


Figure 22: a.) There was no significant relationship between OFC D2 mRNA hybridization and delay discounting. b.) There was no difference in D2 mRNA hybridization in OFC between groups. c.) There was a quadratic relationship between D2 mRNA hybridization in mPFC and delay discounting ($p < .01$). d.) The high and low impulsivity groups both exhibited greater D2 mRNA abundance in mPFC than the mid group.

revealed that the high impulsive rats had significantly more hybridization in mPFC than mid impulsive rats ($p < .05$), that there was a near significant difference between low impulsive and mid impulsive rats ($p = .07$) and that there was no difference between high and low impulsive rats ($p = .37$).

Delay Discounting and Striatum

There were no significant linear correlations or nonlinear curve estimation analyses between DS or NAcc and delay discounting performance. There were also no effects of impulsivity group on cRNA hybridization in either region, although DS approached significance ($p = .07$; Figure 23).

Effort-based Discounting and Prefrontal Cortex

There were no significant linear correlations between effort-based discounting and D2 cRNA hybridization in OFC or mPFC (*ps* > .29), nor were there any significant relationships based on quadratic curve-fit analysis (*ps* > .31). Finally, there were no effects of effort group on hybridization in OFC or mPFC (*ps* > .35; Figure 24).

Effort-based Discounting and Striatum

There were no significant correlations, non linear curves, or group comparisons between effort and cRNA hybridization in DS or NAcc (*ps* > .47; Figure 25).

The Relationship between D2 cRNA Hybridization and Impulsive Action

There were no significant correlations between cRNA hybridization and impulsive action as assessed in any of the DRL protocols (*ps* > .20). As with the

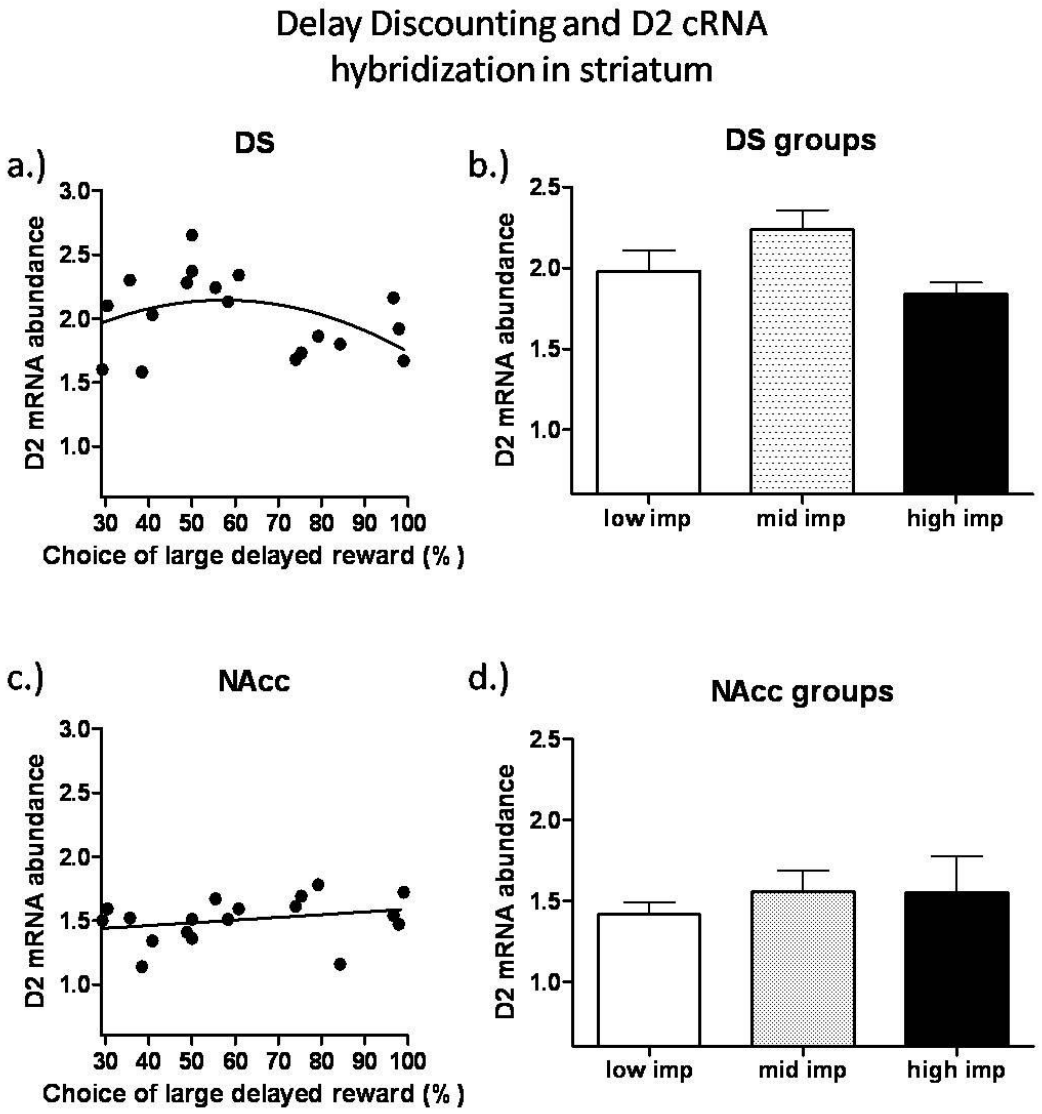


Figure 23: a.) There was no relationship between DS D2 mRNA hybridization and delay discounting. b.) There was no difference in D2 mRNA hybridization in DS between groups. c.) There was no relationship between D2 mRNA in NAcc and delay discounting. d.) There was no difference in NAcc D2 hybridization between groups.

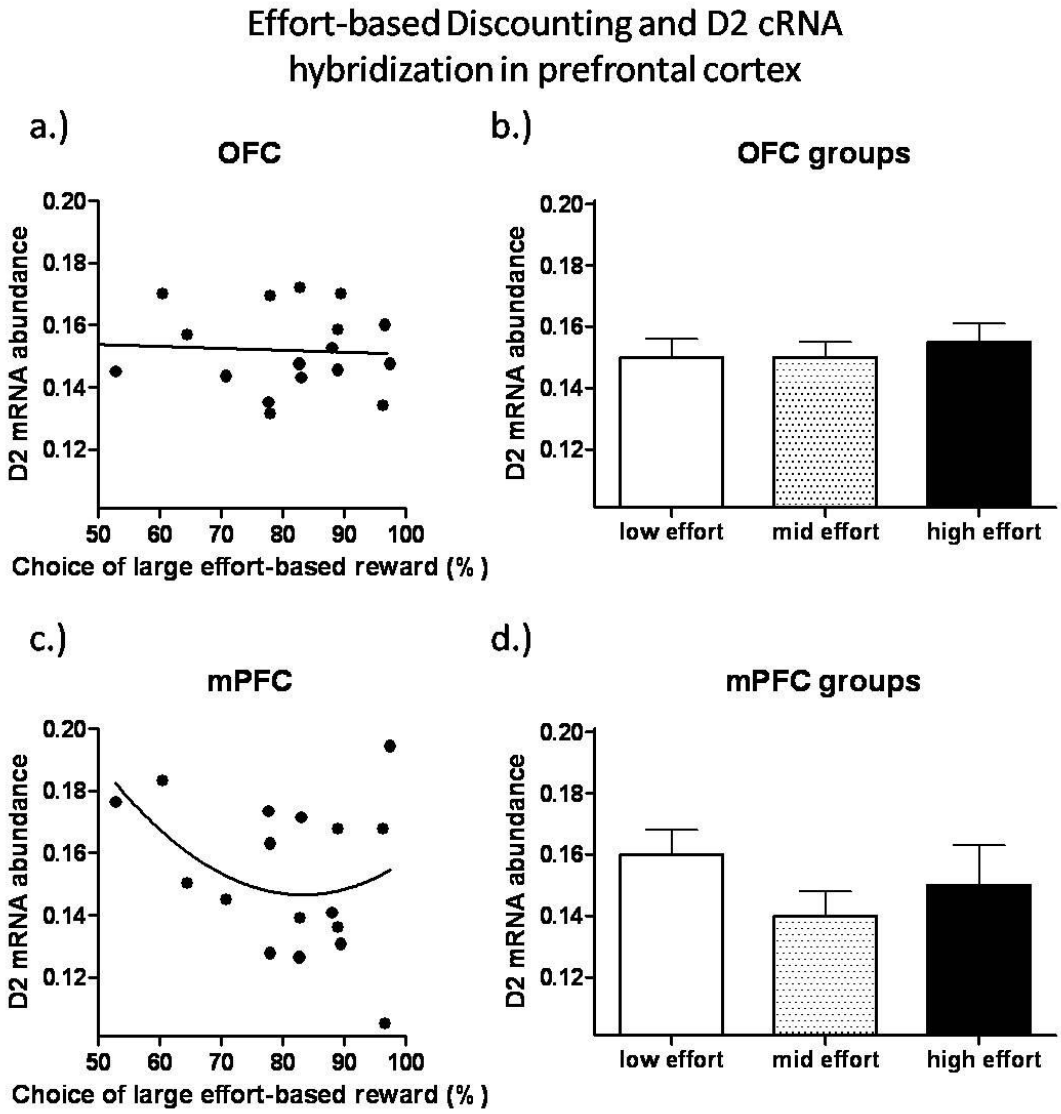


Figure 24: a.) There was no relationship between OFC D2 mRNA hybridization and effort-based discounting. b.) There was no difference in D2 mRNA hybridization in OFC between groups. c.) There was no relationship between D2 mRNA in mPFC and effort-based discounting. d.) There was no difference in mPFC D2 hybridization between groups.

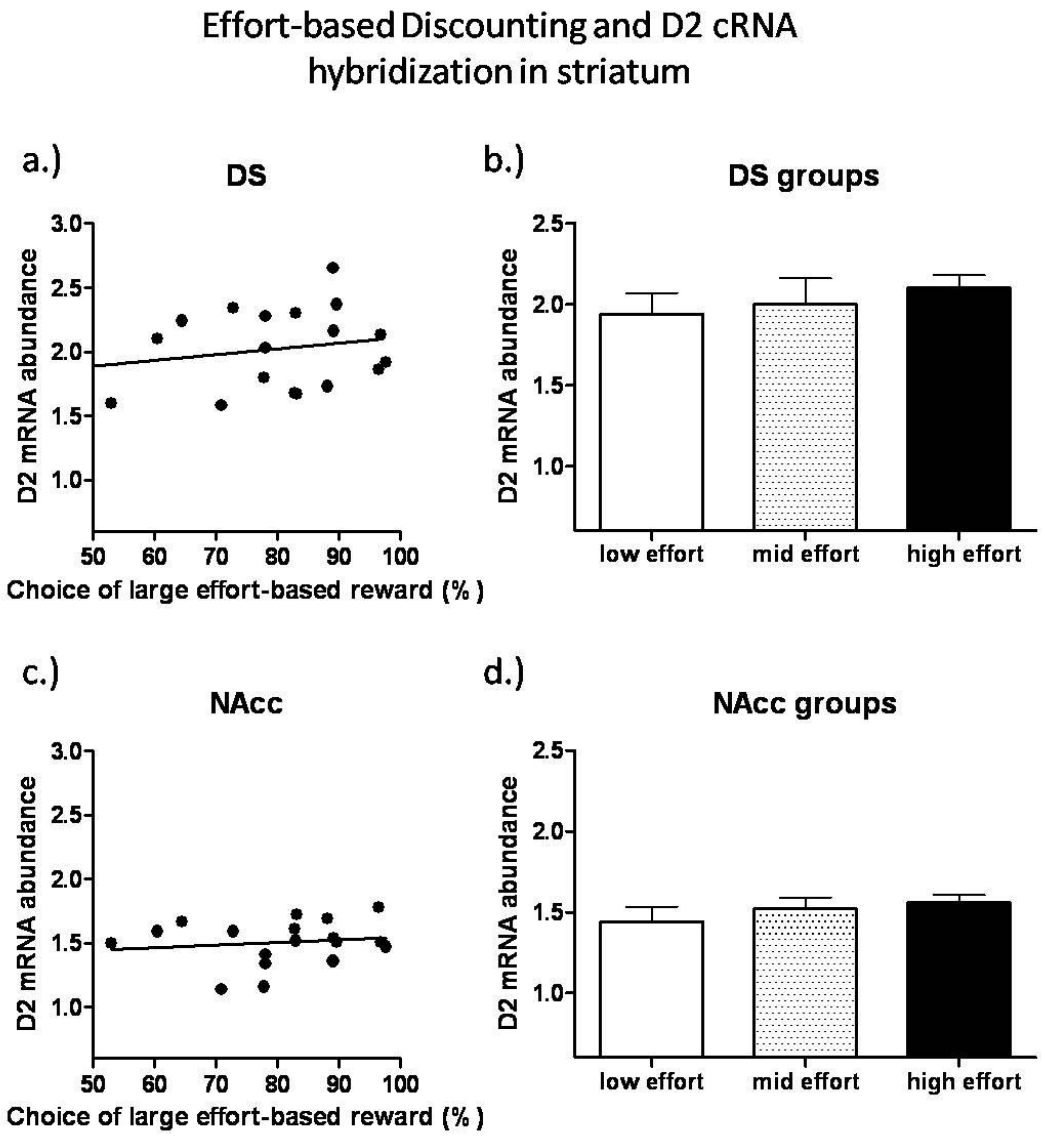


Figure 25: a.) There was no relationship between DS D2 mRNA hybridization and effort-based discounting. b.) There was no difference in D2 mRNA hybridization in DS between groups. c.) There was no relationship between D2 mRNA in NAcc and effort-based discounting. d.) There was no difference in NAcc D2 hybridization between groups.

decision-making tasks, it was expected that the relationships between factors may be non-linear; therefore, curve estimation analysis was utilized to test for the presence of a quadratic relationship between variables. There was a significant quadratic relationship between DRL-20 performance and hybridization in mPFC that resembled a modified inverted U curve ($F_{(2,14)} = 4.13$, $p < .05$; Figure 26). There were no other significant quadratic relationships between any other brain regions and any other measures of impulsive action (*ps* > .23).

As earlier, rats were split evenly into three groups based on task performance and ANOVAs were performed comparing hybridization between groups. There was a significant difference between groups in NAcc hybridization signal such that rats in the high impulsive action group displayed reduced cRNA hybridization compared to rats with either mid- or low- impulsive action ($F_{(2,15)} = 5.07$, $p < .05$; Figure 27). Post hoc tests demonstrated that there was a significant difference between high and mid impulsive groups ($p < .01$), a near significant difference between high and low impulsive groups ($p = .08$), and no difference between high and mid impulsive groups ($p = .21$). There were no other differences in cRNA hybridization between groups for the other brain regions (*ps* > .19).

The Relationship between D2 cRNA Hybridization and Other Behavioral Tasks

Motivation

There were significant negative linear correlations between mPFC D2

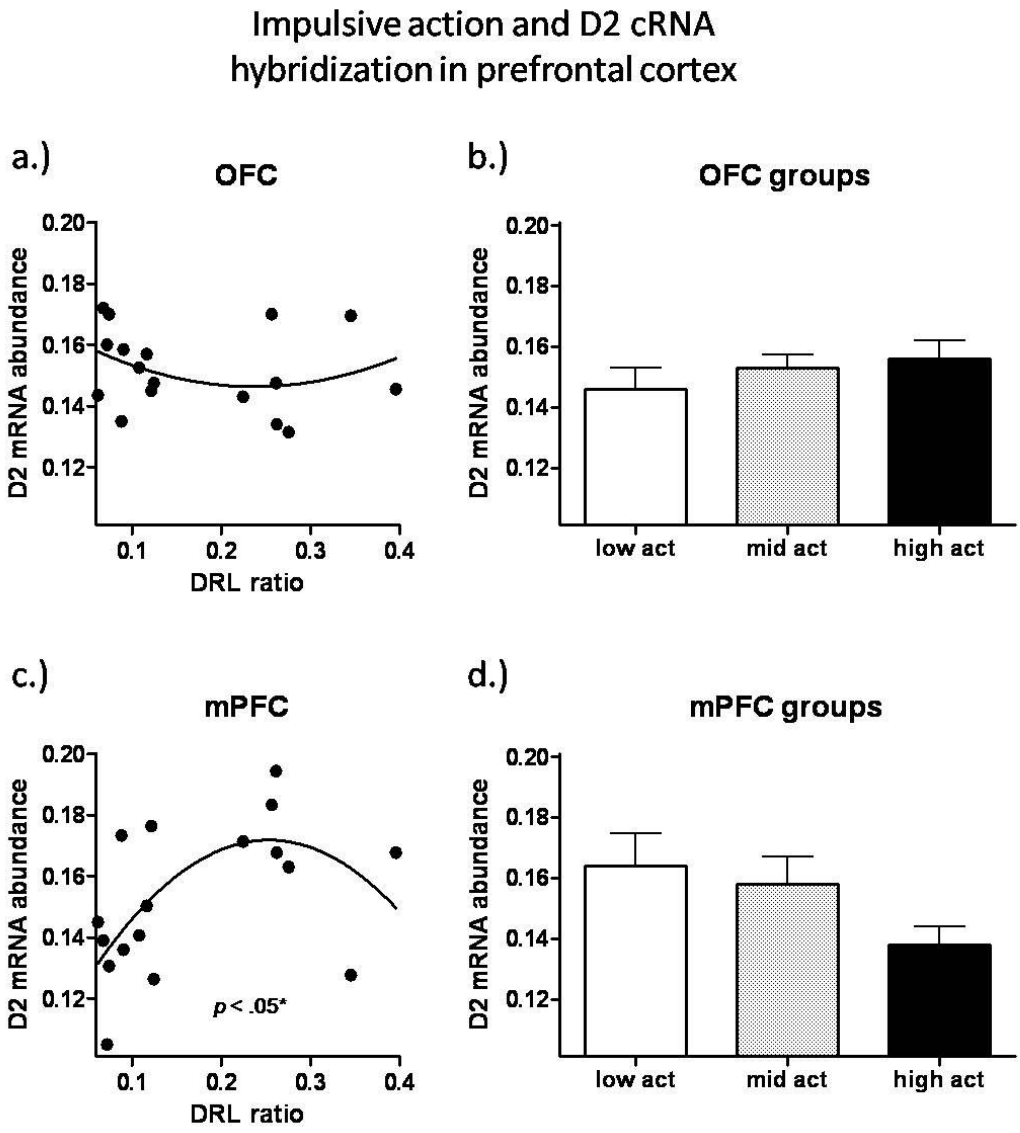


Figure 26: a.) There was no relationship between OFC D2 mRNA hybridization and impulsive action. b.) There was no difference in D2 mRNA hybridization in OFC between groups. c.) There was a significant inverted U-shaped relationship between D2 mRNA in mPFC and impulsive action (*p* < .05). d.) There was no difference in mPFC D2 hybridization between groups.

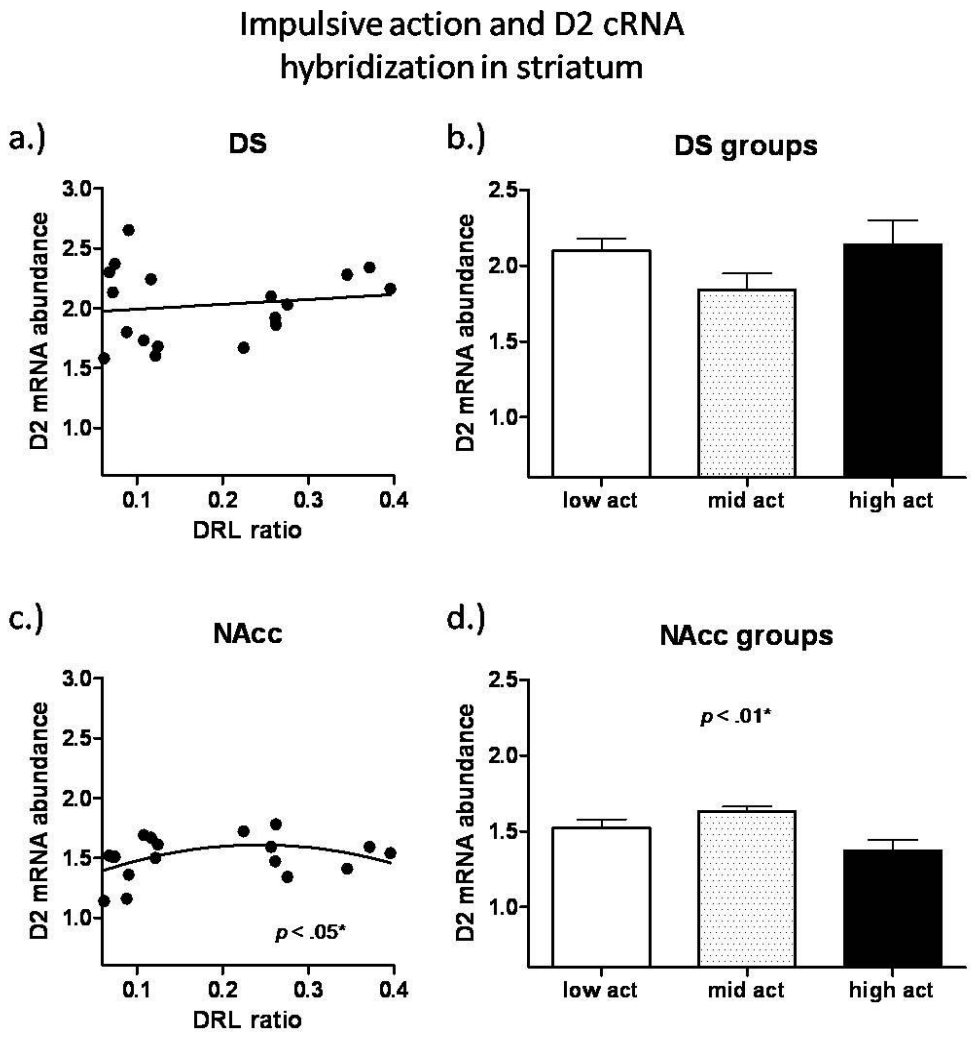


Figure 27: a.) There was no relationship between DS D2 mRNA hybridization and impulsive action. b.) There was no difference in D2 mRNA hybridization in DS between groups. c.) There was a significant nonlinear relationship between D2 mRNA in NAcc and impulsive action ($p < .05$). d.) There was a difference in NAcc D2 hybridization between groups such that the high impulsive action group displayed less D2 mRNA hybridization than the mid impulsive action group ($p < .01$).

cRNA hybridization and both 2.5 and 20% sucrose consumption (*rs* > .64, *ps* < .01), and there was a near-significant negative correlation between 10% sucrose consumption and mPFC hybridization (r = -.54, p = .03). The data indicate that D2 mRNA in mPFC is associated with lower levels of consummatory motivation for sucrose. There were no significant correlations between hybridization in any other region and any measures of motivation (*ps* > .07).

Pain Tolerance

There were near significant correlations between OFC hybridization and both motor and vocalization shock thresholds (*rs* > .57, *ps* > .02), indicating that higher D2 mRNA levels in OFC are associated with a higher pain tolerance in response to shock. In contrast, there was a near significant negative correlation between tail flick latency and OFC D2 hybridization (r = -.55, p = .02), suggesting that higher D2 levels in OFC may also be associated with reduced tolerance to pain as assessed by the tail flick test. A similar near-significant negative correlation was found between tail flick latency and dorsal striatum D2 hybridization (r = -.49, p = .04). There were no other significant correlations between measures of pain tolerance and D2 hybridization (*ps* > .11).

Anxiety

There were significant negative correlations between D2 hybridization in mPFC and both horizontal activity and total locomotion in the open field test (*rs* > -.61, *ps* < .01), indicating that increased D2 in mPFC is associated with decreased general locomotor activity. There was also a near significant positive correlation between mPFC hybridization and time spent in the center of the open

field chamber (r = .55, p = .02), suggesting that higher mPFC D2 hybridization is associated with lower levels of general anxiety.

DISCUSSION

Experiment 1: The Relationship between Risky Decision-making and Other Behavioral Measures

Rats were characterized in risky decision-making, then three other cost-benefit decision-making tasks: probabilistic, delay, and effort-based discounting. Importantly, there were no correlations between risky decision-making and these other tasks. Rats demonstrated the ability to shift reward preference in accordance with changes in discounting factors, resulting in distinct discounting curves and distributions of individual performance across all tasks. The only significant relationship among performance on these tasks observed was between delay discounting and effort-based discounting, such that rats with a high tolerance for delays (lower levels of impulsive choice) were also willing to exert more effort for large rewards. The lack of a relationship between probabilistic discounting and delay discounting was as expected, as this non-relationship had been observed previously by our lab (Simon et al., 2009), and there is considerable evidence suggesting that delay and probabilistic discounting utilize distinct neural pathways (Cardinal 2006; Green et al. 1999; Kheramin et al. 2003; Weber & Huettel 2008). In contrast with previous data, however, there was no relationship between probabilistic discounting and risky decision-making (Simon et al., 2009), although it is important to note that the variance observed in probabilistic discounting was considerably less here than in Simon et al., which may have contributed to the lack of an observed correlation

(especially when considering the high degree of variance observed in the risky decision-making task).

There were no significant correlations between performance in any decision-making task and behavioral measures of motivation, pain tolerance, or anxiety. The absence of a relationship between pain tolerance and the novel risky decision-making task is of particular importance because this task incorporates an aspect of physical punishment (footshock) that has heretofore not been integrated into any rodent cost-benefit decision-making tasks. Thus, it was possible that the physical response to painful stimuli may be the principal factor mediating choice behavior during this task (rather than risk). However, the complete lack of relationship between any of multiple measures of pain tolerance and risky decision-making indicated that choice was likely not mediated solely by sensitivity to pain, but instead by a separate reward discounting process that reflected willingness to risk punishment.

The lack of a correlation between any measures of motivation and decision-making is somewhat inconsistent with a recent study by Rivalan et al. (2009). In this study, rats that demonstrated maladaptive decision-making on a rat model of the Iowa Gambling Task also showed greater levels of appetitive motivation as assessed by a progressive ratio instrumental task. In the current study, there were no relationships between any forms of cost-benefit decision-making and progressive ratio performance. This is likely a result of subtle task differences: in Rivalan et al., the decision-making task included specific alternatives that were optimal in comparison to the other choices (based on total

food received), which classifies this task as a measure of ability to assess outcomes within working memory. This differs from the majority of the cost-benefit decision-making tasks outlined in this study, which instead offered choices between two options that were both associated with varying costs and benefits (without a clear “correct” option). Therefore, the differences between the constructs being measured by these distinct tasks offer an explanation for the differences in observed correlations.

Several relationships were observed between measures of impulsive action and cost-benefit decision-making. There was a relationship between delay discounting and impulsive action as assessed by the DRL task such that rats with high levels of impulsive choice also demonstrated high impulsive action. These data provide evidence that different aspects of impulsivity may reflect a unitary construct. Indeed, increases in both impulsive action and impulsive choice have been found to result from extended psychostimulant access (Fletcher et al. 2007; Peterson et al. 2003; Roesch et al. 2007b; Simon et al. 2007), and high levels of both of these traits predict several aspects of drug self-administration (Anker et al. 2009; Belin et al. 2008; Dalley et al. 2007; Perry et al. 2005; Perry et al. 2008). However, there is also considerable evidence from pharmacological and lesion studies that these different aspects of impulsivity are mediated by distinct mechanisms (Pattij & Vanderschuren 2008; Voon et al. 2010; Winstanley et al. 2004a). The discrepancy between the results of this experiment and these data may be a result of task differences, as this study utilized the DRL to measure impulsive action while the others used the 5-choice serial reaction time task.

While the DRL has been frequently used to measure impulsive action (Evenden & Ryan 1996; Sokolowski & Salamone 1994; Uslaner & Robinson 2006), it is also possible that this task is reflective of time assessment (Orduña et al. 2009). This could potentially explain the relationship found between DRL and delay discounting, as rats with abnormal timing ability may also demonstrate an overestimation of delays, leading to an increase in the discounting of delayed rewards.

There was also a correlation between the risky decision-making task and DRL performance such that rats with a preference for the risky reward demonstrated higher levels of impulsive action. One potential explanation for this result is that impulsive action and risky decision-making are governed by similar brain pathways and pharmacological substrates, an assumption that will require further delineation of the biological circuitry underlying risky decision-making utilizing lesion studies to confirm. Another possibility is that choice of the risky reward is a direct result of a highly impulsive behavioral phenotype. The risky reward is not accompanied by punishment during the opening trials of the risky decision-making task, then subsequently becomes associated with increased risk of punishment as the task progresses. Impulsive action can be defined as an inability to withhold a prepotent response; rats that continue to select the risky reward may be responding in this fashion because of an inability to withhold the previously “safe” choice of the large reward even after it becomes a “risky” option. However, there is some evidence to the contrary, as impulsive action does not predict choice of the large reward during the probabilistic discounting

task. Had cost-benefit decision-making response patterns in general been governed by prepotent responding in impulsive rats, then rats characterized as impulsive on the DRL task would have been expected to continue to select the large, initially advantageous reward throughout this task (even when selection produces 0% reinforcement during the final block of each session).

In summary, risky decision-making in rats cannot be predicted by general behavioral processes such as motivation, anxiety, and pain tolerance. Therefore, at least to some degree, the integration of rewards with risk of physical punishment in order to guide choice behavior can be measured as a distinct behavioral construct. Additionally, the discounting of rewards via probabilistic punishment appears to be regulated differently from the discounting of rewards due to delay, probability of reward omission, or effort required, as reward preference shifted differently according to the discounting factor being utilized, and there were no correlations between risky decision-making and the other three tasks. Risky choice was correlated with impulsive action, suggesting that these processes may be mechanistically similar, or that they may interact in some fashion (i.e., impulsive rats may be more likely to select an option that had been associated with reward earlier without fully considering the degree of risk involved).

Experiment 2: The Effects of Systemic Dopaminergic Manipulation on Risky Decision-making

Systemic amphetamine administration produced a dose-dependent decrease in risk-taking during the risky decision-making task, shifting rats'

preference toward the 'safe' reward. This effect (which resulted in less overall food availability) was likely not an artifact of amphetamine induced suppression of food intake (Wellman et al. 2009), because previous work in our lab demonstrated that neither 1- nor 24-h periods of free feeding before testing had an effect on reward choice, although 24-h free feeding did increase the number of trials omitted (an effect that was also observed under amphetamine) (Simon et al. 2009). Another possibility is that the increased preference for the small, safe reward induced by amphetamine was a result of increased pain sensitivity to footshock. This explanation seems unlikely for three reasons: first, amphetamine has been characterized as an analgesic agent (Connor et al. 2000; Drago et al. 1984). If pain sensitivity were indeed the critical mediator of reward selection in this task, rats given amphetamine would be expected to find the shock less aversive and shift their preference toward the large reward as a result of a higher pain threshold. Second, amphetamine did not alter reactivity (as assessed by locomotion) during the footshock, which can be used as a behavioral marker for pain/shock sensitivity (Chhatwal et al. 2005). Third, data from Experiment 1 showed that reward choice was not associated with various measures of pain tolerance, indicating that the level of pain induced by the footshock is likely not the critical factor mediating reward choice.

While amphetamine elicits an increase in synaptic dopamine, serotonin, and norepinephrine, our experiments focused on the dopaminergic system, as amphetamine induces a larger increase in dopamine activity than other neurotransmitter systems (Azzaro & Rutledge 1973; White & Kalivas 1998) (to be

discussed further below). Amphetamine does not act on specific dopaminergic receptor subtypes, but instead causes a general increase in synaptic dopamine, which in turn acts on all dopamine receptors in a nondiscriminatory fashion. To address which receptor subtypes may specifically mediate risky choice, the effects of dopaminergic agents specific to either D1 or D2 dopamine receptors on risk were observed. Neither SKF81297, a D1 dopamine receptor agonist, nor SCH23390, a D1 dopamine receptor antagonist, influenced risky decision-making behavior. Furthermore, co-administration of SCH 23390 with amphetamine was not sufficient to block the amphetamine-induced attenuation in preference for the risky reward. Therefore, it seems that D1 dopamine receptors do not directly mediate risky decision-making behavior in rats.

In contrast, bromocriptine, a D2 receptor agonist, caused a decrease in risky choice during the risky decision-making task. This effect was dose dependent, and qualitatively resembled the effects of acute amphetamine on risky decision-making behavior. Eticlopride, a D2 antagonist, did not affect risky decision-making behavior. Importantly, however, coadministering eticlopride with amphetamine blocked amphetamine's effects on risky choice without blocking amphetamine's general locomotor enhancing effects. These data provide evidence that D2 dopamine receptors are necessary for amphetamine to exert an influence on risky choice, and sufficient to alter risky choice independently from D1 activation.

It is difficult to discern the mechanism of action of drugs that target the D2 dopamine receptor, as two distinct manifestations of D2 receptors have been

identified. One of these acts as a standard post-synaptic receptor, while the other is located presynaptically and functions as an autoreceptor that inhibits dopamine release from the terminal (De Mei et al. 2009). Despite the functional differences between these receptor types, they possess similar pharmacodynamic properties, and thus would have similar affinities for D2 agonists or antagonists (Emilien et al. 1999). Therefore, a D2 agonist exerts opposing effects on post synaptic neurons, simultaneously binding to autoreceptors (thereby reducing available dopamine in the synapse) and directly binding to postsynaptic receptors. Conversely, a D2 antagonist would increase synaptic dopamine through blockade of the autoreceptors, while also blocking postsynaptic receptors. One method that may be utilized to delineate the effects of pre- and post-synaptic D2 receptors on risky choice would be to utilize genetic knockout subjects, as knockouts specific to either D2 isoform have been developed (Centonze et al. 2004; Lindgren et al. 2003).

The shift toward risk aversion induced by amphetamine and bromocriptine seems somewhat counterintuitive, as structures such as ventral tegmental area and NAcc that are profoundly impacted by dopaminergic drugs have frequently been implicated in aspects of reward-seeking/motivation (Berridge 2007; Everitt & Robbins 2005; Kelley et al. 2005; Salamone et al. 1994; Wise 2009), and intracranial infusions of amphetamine into NAcc enhance reward motivation (Phillips et al. 2003). However, the same areas implicated in reward also appear to be involved with emotional reactions to aversive stimuli (Carlezon & Thomas 2009; Liu et al. 2008; Setlow et al. 2003). Thus, it is

possible that amphetamine-induced enhancements in dopamine transmission increase the ability of aversive stimuli to control behavior (rather than solely enhancing the influence of rewarding stimuli), which could explain the amphetamine-induced shift in reward choice away from the large, risky reward. This explanation is consistent with previous findings showing that acute amphetamine administration at doses similar to those used here increased the degree to which rats avoided making a response that produced an aversive conditioned stimulus previously associated with footshock (i.e., amphetamine increased control over responding by the aversive conditioned stimulus (Killcross et al. 1997)).

Interestingly, humans demonstrate “paranoid” patterns of behavior following amphetamine exposure during which they report accentuated sensitivity to aversive events, memories, and cues (Dawe et al. 2009; Ellinwood & Cohen 1971; Moutoussis et al. 2007). This hypersensitivity produces avoidant and overly cautious behavior, which may be analogous to the increase in risk-averse choice seen in amphetamine-exposed rats performing the risky decision-making task. Thus, this task may offer a potential animal model (albeit solely based on a behavioral phenotype) of amphetamine-induced “paranoid” behavior.

The data obtained in this experiment are consistent with Zeeb et al. (2009), in which the novel Rat Gambling Task was used to measure risky choice in rats during which the risky option was accompanied by a possibility of a “time out” during which no food was available (resulting in less overall food availability over the course of the session). The multiple options presented during this task

included an excessively risky option with large rewards and punishment, a safe option with a small magnitude of both reward and punishment, and a moderately risky option that ultimately produced the largest amount of net reward throughout the session (which rats typically preferred upon acquisition of the task). Interestingly, acute amphetamine caused an increase in selection of the safest option and a reduced selection of the moderate-risk option. Therefore, amphetamine induced an increase in risk aversion that resulted in a sub-optimal amount of available reward. Thus, risk of physical punishment and risk of "time-outs" during which rewards and active participation in the task are unavailable seem to both be attenuated by amphetamine.

The attenuation in risk-taking induced by amphetamine and bromocriptine differed from effects observed on a probabilistic decision-making task, which measured risk of reward omission rather than risk of punishment. St. Onge and Floresco (2009) reported that both amphetamine and bromocriptine increased risky choice in a probabilistic discounting task, which is contrary to the decrease in risky choice found here. This dissociation was likely a result of the difference in discounting factors associated with the large reward between these tasks: dopamine neurotransmission appears to enhance the salience of a punishing factor (footshock) (Killcross et al. 1997, Simon et al. 2009), thereby biasing behavior away from the large reward in the risky decision-making task because of the enhanced salience of the threat of punishment. On the other hand, in the probabilistic discounting task, dopamine neurotransmission may bias behavior toward the large reward because the discounting factor is not physical

punishment, but is instead probability of omission. Therefore, rats would be more likely to “gamble” due to the enhanced salience of the food reward and the relatively smaller salience of the food omission. Another possibility is that the integration of distinct discounting factors (reward omission vs. punishment) with rewards may utilize separate neural and pharmacological mechanisms, with these distinct systems responding differently to augmented dopamine activity. Also contradictory to previous data were the lack of effects of the D2 dopamine receptor antagonist eticlopride on risky decision-making. D2 antagonists had previously been shown to decrease risky choice in probabilistic discounting (St. Onge et al. 2009) and the Rat Gambling Task (Zeeb et al. 2009). Again, this discrepancy can likely be attributed to differences between tasks.

An important factor to consider is that the effects of amphetamine may be partially mediated by neurotransmitters other than dopamine, as amphetamine also enhances serotonin transmission (Azzaro & Rutledge 1973; Holmes & Rutledge 1976). Serotonin has been found to mediate risky behavior (Juhasz et al. in press) as well as other forms of cost-benefit decision-making (Pattij & Vanderschuren 2008; Winstanley et al., 2005), and therefore could be responsible for the amphetamine induced attenuation of risky decision-making. However, this is improbable for two reasons: first, administration of the D2 agonist bromocriptine, which has no direct effect on serotonin transmission, was sufficient to replicate the behavioral effects of amphetamine on risky choice (See Figures 9 and 12). Second, in a previous study, the effects of acute cocaine exposure, which has much greater affinity for the serotonin transporter than

amphetamine (White & Kalivas 1998), did not induce an attenuation of risk, but instead seemed to produce an impairment in probability recognition (Simon et al. 2009) . Therefore, it is unlikely that amphetamine's effects on risky choice are solely a result of enhanced serotonin availability (although it seems likely that serotonin is involved with risky decision-making behavior in some fashion, and this issue merits further exploration). Norepinephrine availability in the synapse is also enhanced by amphetamine, and more research is necessary to clarify the specific role of this neurotransmitter in rat models of risky choice.

In conclusion, amphetamine causes a dose-dependent decrease in risky choice behavior. This effect can be duplicated using the specific D2 receptor agonist bromocriptine, and can also be blocked by coadministering the D2 antagonist eticlopride with amphetamine. In contrast, the D1 agonist SKF81297 is not sufficient to produce this effect, and co-administration of the D1 antagonist SCH23390 with amphetamine does not block the effect. Therefore, it can be surmised that D2 receptors play a critical role in the mediation of risky choice behavior, and offer a potential therapeutic target for psychopathologies characterized by excessive risk-taking.

Experiment 3: The Relationship between Dopamine Receptor mRNA and Risky Decision-making

Prefrontal Cortex Dopaminergic Mediation of Risky Decision-making

The results obtained in Experiment 2 suggested that D2 dopamine receptors are particularly involved in risky decision-making behavior. In Experiment 3, the relationship between baseline D2 receptor expression in

various brain regions and risky decision-making was assessed. A nonlinear relationship was uncovered between risky choice and D2 receptor expression in OFC, a region implicated in representation of both reward and punishment as well as the integration of information to guide complex decision-making processes (McCabe et al. ; Morrison & Salzman 2009; Rolls 2004; Schoenbaum et al. 2006; Schultz 2007). Rats that demonstrated either a profound preference for the risky reward **or** an aversion toward risky choice both displayed higher levels of D2 expression in OFC than rats that displayed moderate levels of risk-taking. Therefore, higher levels of D2 receptor expression in OFC seem to be related to "behavioral extremes" such that rats exhibit a strong preference (or aversion) toward a specific option and rarely shift reward preference despite changes in contingencies, whereas rats with lower levels of D2 expression were better able to modify their behavior according to the changing response-outcome contingencies.

Curiously, D2 expression in mPFC adheres to an opposite trend, with the moderately risky behavioral phenotype being associated with a higher level of D2 mRNA than both the risk-taking and risk-averse phenotype. This inverted U-shaped pattern is in stark contrast to the U-shaped pattern of results in OFC, and suggests that the two structures mediate risky choice in a dissociable fashion. It is conceivable that risky choice is a function of the relationship between structures, as these structures interact through reciprocal connections (Moghaddam & Homayoun 2007). OFC and mPFC form a circuit involved in the prediction and evaluation of the economic value of outcomes (OFC) and the

execution of the appropriate action (mPFC), and opposing patterns of plasticity between these structures have been observed during encoding of appetitive information (Moghaddam & Homayoun 2007; Rolls & Grabenhorst 2008). Importantly, this pattern of dissociation between cortical structures extends to dopaminergic transmission. In humans with Parkinson's Disease, L-DOPA treatment impaired performance in an OFC-dependent gambling task, but improved performance in a set shifting task that relies on dorsolateral prefrontal cortex, a structure that appears to be functionally analogous to rodent mPFC (Brown & Bowman 2002; Cools et al. 2003; Uylings et al. 2003). Even more relevant is the fact that the D2 agonist bromocriptine produced opposite effects on cognitive tasks mediated by these two different prefrontal cortical regions, impairing OFC-dependent reversal learning and enhancing dorsolateral prefrontal cortex dependent working memory (Mehta et al. 2001). Therefore, the heterogeneity of dopaminergic (and specifically D2-related) neurotransmission within different prefrontal cortical structures found here is not without precedent. The interaction between these different but interconnected systems may be a critical component of the development of behavioral biases either toward or away from risky choice. Studies utilizing contralaterally placed lesions or electrophysiological recording could be used to elucidate the specific nature of this functional relationship.

Striatal Dopaminergic Mediation of Risky Decision-making

A relationship was also observed between D2 receptor expression in DS and risky decision-making. As with OFC, this relationship was a non-linear

function, although the pattern of results was different; in that risk-averse rats displayed greater D2 mRNA than both moderate-risk and risk-taking rats. The involvement of DS in risky decision-making is particularly interesting, as DS has been traditionally associated with habitual learning and memory rather than complex behavior based on multiple cues and contingencies (Everitt et al. 2008; Packard 2009). The current findings, along with recent sentiment that DS may in fact contribute to action selection and flexible decision-making (Balleine et al. 2007; Johnson et al. 2007), suggest DS as a region that may play a pivotal role in the cost-benefit decision-making process.

These data complemented the results obtained in Experiment 2, as enhanced D2 receptor expression in DS was associated with attenuated risky choice. This suggests that enhanced D2 receptor binding in DS may be responsible for amphetamine and bromocriptine's effects on risky decision-making. However, it is important to note that both OFC and mPFC, in which D2 levels also were associated with risk, are functionally connected to DS as well as each other (Berendse et al. 1992; Ragozzino 2007). Therefore, it is more likely that a dopamine-modulated circuit consisting of these structures (and likely others as well) contributes collectively to the risky decision-making process.

NAcc D2 expression was not associated with risky decision-making performance. This is somewhat surprising because NAcc is involved with the representation of both rewarding and aversive outcomes (Schoenbaum & Setlow 2003; Setlow et al. 2003), both of which are factors in the risky decision-making task. Additionally, NAcc has been found to mediate performance in several other

cost-benefit decision-making tasks (Cardinal & Howes 2005; Cardinal et al. 2001; Hauber & Sommer 2009). While this study provides evidence that baseline D2 receptor expression in NAcc does not strongly influence risky decision-making, further work is necessary to determine if an intact NAcc is required for risky decision-making, or if other neurotransmitters (or dopamine receptor subtypes) within NAcc mediate risky choice.

The Relationship between D2 Receptors and Other Forms of Cost-benefit Decision-making

Probabilistic discounting performance displayed a modest negative correlation with D2 expression in OFC, indicating that enhanced preference for higher risk of reward omission (i.e., "gambling" behavior) was associated with lower levels of D2 receptor availability. This is somewhat surprising, as mPFC rather than OFC has typically been implicated in probabilistic discounting performance (Cardinal 2006; St. Onge & Floresco In Press). However, it is important to note that the observed correlation and differences between groups were both contingent on the probabilistic discounting performance of three specific subjects, all of which exceeded 2 standard deviations from the group mean (Figure 19). Thus, replication using more subjects may be necessary before making strong inferences using these data.

The lack of a relationship between OFC or NAcc D2 expression and delay discounting is somewhat surprising, as OFC and NAcc rather than mPFC have traditionally been implicated in delay discounting (Cardinal et al. 2001; Roesch et al. 2006; Rudebeck et al. 2006; Winstanley et al. 2004b). Dopamine transmission

has also been associated with impulsivity and delay perception (Cardinal et al. 2000; Winstanley et al. 2005), and dopamine in OFC has specifically been associated with impulsive choice, as dopamine depletion localized to OFC decreases impulsive choice (increasing choice of the larger delayed reward) (Kheramin et al. 2004). It is possible that delay discounting is mediated by other dopaminergic receptor subtypes, although it has been observed that systemic D2 rather than D1 antagonists increase impulsive choice (Wade et al. 2000). Another possibility is that, while extreme shifts in dopamine neurotransmission induced by direct or indirect agonists can influence impulsive choice, the relatively small baseline differences in D2 expression in OFC and NAcc do not produce substantial variations in impulsive choice.

In contrast with the results in OFC and NAcc, delay discounting performance was associated with D2 expression in mPFC, such that rats with either high or low levels of impulsivity both displayed higher expression of D2 mRNA than rats with moderate levels of impulsive choice. While not as well studied as OFC with regard to delay discounting, there are recent data showing that mPFC inactivation can increase impulsive choice (Churchwell et al. 2009). Additionally, enhancements in dopamine and dopamine metabolites have been observed in mPFC during delay discounting, although this same increase occurred in a yoked group, implying that dopamine in mPFC may mediate motivational processes rather than reward choice. As mentioned earlier mPFC is reciprocally connected to OFC (Moghaddam & Homayoun 2007); therefore, D2

receptor expression in mPFC may affect impulsive choice indirectly by influencing OFC function, possibly via dopaminergic mechanisms.

The Relationship between D2 Receptors and Other Behavioral Measures

A relationship was uncovered between impulsive action and D2 expression in NAcc such that highly impulsive rats expressed less D2 mRNA than rats with lower impulsivity. This replicated a previous study which reported that reduced D2 receptor availability predicts a high impulsive phenotype in rats (Dalley et al. 2007). Additionally, a relationship was also observed between mPFC D2 expression and impulsive action such that highly impulsive rats expressed less baseline D2 receptor mRNA in mPFC than low impulsive rats. This is not surprising, as lesions of mPFC induce a variety of deficits in impulsive action, including excessive premature responding (the measure utilized here). It is likely that NAcc and mPFC act in concert to regulate impulsive action, as intracranial infusions of the D2/D3 antagonist sulpiride into NAcc ameliorated the mPFC lesion deficits (Pezze et al. 2009).

A negative linear relationship was revealed between mPFC D2 expression and sucrose consumption such that rats with increased D2 expression in mPFC demonstrated lower consummatory motivation. This seems counterintuitive, as the D2 agonist quinpirole enhances drinking behavior to a near-compulsive degree (Amato et al. 2007; Fraiolo et al. 1997). Additionally, it has been observed that during feeding behavior, a dopamine efflux occurs in both mPFC and NAcc that gradually decreases as satiety ensues (Ahn & Phillips 2002). Because we observed here that increased D2 expression in mPFC predicts lower levels of

feeding, it is possible that dopamine signaling in mPFC during consumption may be exerting an inhibitory influence, possibly mediated by D2 receptors.

A negative correlation was discerned between D2 expression in mPFC and multiple measures of spontaneous locomotion. The finding that high baseline levels of D2 expression predict lower locomotion is not entirely surprising; direct infusions of the D2 agonist quinpirole into mPFC blocks both acute and sensitized cocaine-induced locomotor enhancement (Beyer & Steketee 2002). Additionally, a contributing factor to drug induced locomotor sensitization (which results in increased locomotor responses to drugs and novel stimuli) is a decrease in D2 receptor availability, which reduces the mPFC's inhibitory influence over NAcc (Kalivas et al. 2005). Therefore, an increase in the inhibitory power of mPFC via higher D2 receptor availability (which, presumably, would result from enhanced D2 expresssion) would be expected to reduce spontaneous locomotor activity.

Experiment 3 Summary

Relationships between D2 dopamine receptor expression and risky decision-making behavior were revealed in multiple brain regions. In OFC, D2 expression was greater for both risk-taking and risk-averse rats than in moderately risky rats. Conversely, in mPFC D2 expression was higher for moderate risky rats and lower for the other groups. The opposing patterns of results between interconnected regions resemble the heterogeneity observed between these structures in other contexts (Moghaddam & Homayoun 2007; Robbins 2005) , and further experimentation is necessary to delineate how these

reciprocal circuits interact to guide risky behavior. Importantly, the nonlinear patterns of results observed in both OFC and mPFC were not entirely surprising, as dose-response curves to pharmacological treatment (including dopamine) during prefrontal cortical reliant tasks are often manifested as U- or inverted U-shaped behavioral trends (Robbins 2005).

A different pattern of results was observed in DS, with the risk-averse group displaying a higher abundance of mRNA expression than the other, riskier groups. These data coincide with the results from Experiment 2, during which rats administered the D2 agonist bromocriptine exhibited a profound attenuation in risky choice. Thus, it is a possibility that DS is the brain region most directly affected by bromocriptine (and amphetamine) that mediates the shift away from risky choice. These data are particularly exciting, as DS has largely been overlooked as a substrate of cost-benefit decision-making.

Relationships were also observed between D2 expression in OFC and probabilistic discounting, as well as between D2 expression in mPFC and delay discounting. Importantly, rats that demonstrated high impulsive action also displayed reduced D2 expression in NAcc, which was theoretically consistent with Dalley et al. (2007).

SUMMARY AND CONCLUSIONS

In Experiment 1, evidence was provided that risky decision-making behavior in rats exists as a behavioral trait separate from general motivation, anxiety, pain tolerance, or other decision-making processes. The risky decision-making task has been shown to be reliable (Simon et al. 2009), and groups of rats consistently display a widespread range of performance, which renders this task especially useful for examining the biological substrates underlying individual differences (as in Experiment 3). These data support the utility of the risky decision-making task for further study of both the cause of and treatments for excessive, maladaptive risky choice.

Experiment 2 demonstrated that acute amphetamine can attenuate risky choice, and that D2 dopamine receptors are necessary and sufficient for this effect. Experiment 3 expanded on the finding that D2 receptors mediate risky behavior by comparing baseline levels of D2 mRNA expression with performance in the risky decision-making task. Risky decision-making behavior predicted levels of D2 mRNA hybridization in both orbitofrontal cortex and medial prefrontal cortex, although these brain regions displayed opposite trends, with orbitofrontal cortex D2 hybridization showing a U-shaped relationship with risky choice, and medial prefrontal cortex D2 hybridization resembling an inverted U-curve. Additionally, increased levels of D2 mRNA in dorsal striatum were observed in risk-averse rats in comparison to risk-taking rats. These data suggest the involvement of a corticostriatal circuit in risky decision-making.

These experiments provide a foundation for an understanding of how dopaminergic transmission acts to regulate risky and impulsive behavior. This is especially important because disorders such as Parkinson's disease, Dopamine dysregulation syndrome, and Restless Leg Syndrome require direct or indirect dopamine agonist treatment, and this treatment often induces near compulsive levels of risky and impulsive behavior (Antonini & Cilia 2009; Pourcher et al. ; Wolters et al. 2008). Risk assessment tasks such as the risky decision-making tasks could be utilized to determine by which action these dopaminergic drugs are inducing such behavioral abnormalities, and what measures could be taken to alleviate these problems.

Decision-making is rarely a black and white process: subjectively rewarding outcomes often require some form of expense, be it the requirement of hard work, the possibility of a hazardous outcome, or the relinquishment of other benefits. The risky decision-making task is a particularly effective model of complex decision-making because it adheres to this concept, offering two options without a clear cut "correct" answer. This ambiguous nature of this task is likely accountable for the wide variance observed, with rats' performance ranging from complete avoidance of the risky reward lever to consistent selection of the risky reward regardless of punishment probability. Perhaps the most striking observation from this study was the number of trends in D2 dopamine receptor expression that were shared by both risk-averse and risk-taking rats. It is interesting to consider that, in some situations, an inability to take risks may be equally as maladaptive as excessive risk taking. The risky decision-making task

offers a reliable and valid method of assessing robust individual differences, testing pharmacological agents, and uncovering the neural networks that integrate risks and rewards.

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Education

1996 - 2000	B.A., Major: Biology, Minor: Psychology, Carthage
2002 - 2004	M.S., Department of Experimental Psychology, Western Illinois University
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Selected Honors and Awards

2006	Poster Award for Excellence in Presentation and Scientific Content, *Eighth Conference on the Neurobiology of Learning and Memory*, University of California, Irvine
2007	Selected as Panel Fellow, *Linking Affect to Action: Critical Contribution of the Orbitofrontal Cortex*, Session VII: Orbitofrontal Cortex, Mental Health, and Aging
2008	Individual Ruth L. Kirschstein Predoctoral National Research Service Award, NIDA
2008	Selected to participate in Early Career Investigators Poster Session, NIDA/APA
2008	Society for Neuroscience Chapters Graduate Student Travel Award
2009	Texas A&M Chapter Society for Neuroscience Poster Competition, Second place

Selected Peer-Reviewed Publications (selected from 7 total)

Simon, N.W., Mendez, I.A., & Setlow, B. (2007). Cocaine exposure causes long-term increases in impulsive choice behavior. *Behavioral Neuroscience, 121,* 543-549.

Simon, N.W., Mendez, I.A., & Setlow, B. (2009). Prior amphetamine exposure alters approach strategy during Pavlovian conditioning. *Psychopharmacology, 202*, 699-709.

Simon, N.W., Gilbert, R.J., Mayse, J.D., & Setlow, B. (2009). Balancing risk and reward: A rat model of risky decision-making. *Neuropsychopharmacology, 34,* 2208-2217.