RESEARCH ARTICLE

Cognitive mechanisms underlying decision making involving risk of explicit punishment in male and female rats

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Abstract

Individuals engage in the process of risk-based decision making on a daily basis to navigate various aspects of life. There are, however, individual differences in this form of decision making, with some individuals exhibiting preference for riskier choices (risk taking) and others exhibiting preference for safer choices (risk aversion). Recent work has shown that extremes in risk taking (e.g., excessive risk taking or risk aversion) are not only cognitive features of neuropsychiatric diseases, but may in fact predispose individuals to the development of such diseases. To better understand individual differences in risk taking, and thus the mechanisms by which they confer disease vulnerability, the current study investigated the cognitive contributions to risk taking in both males and females. Rats were first behaviorally characterized in a decision-making task involving risk of footshock punishment and then tested on a battery of cognitive behavioral assays. Individual variability in risk taking was compared with performance on these tasks. Consistent with prior work, females were more risk averse than males. With the exception of the Set-shifting Task, there were no sex differences in performance on other cognitive assays. There were, however, sex-dependent associations between risk taking and specific cognitive measures. Greater risk taking was associated with better cognitive flexibility in males whereas greater risk aversion was associated with better working memory in females. Collectively, these findings reveal that distinct cognitive mechanisms are associated with risk taking in males and females, which may account for sex differences in this form of decision making.

Keywords Decision making · Sex differences · Punishment · Cognition

Introduction

Decision making, or the ability to evaluate options associated with varying rewards and the potential costs associated with these outcomes, is necessary for healthy cognitive functioning. Although most individuals can engage in this calculus and make optimal choices, individuals with certain neuropsychiatric diseases display impaired decision making due to inappropriate weighting of the rewards and risks associated with available options. For example, individuals with substance use disorders tend to undervalue risks of adverse consequences while placing disproportionate value in rewarding outcomes; such an imbalance in value attribution in decision making can consequently contribute to continued drug-seeking and/or relapse after prolonged abstinence (Chen et al., 2020; Gowin et al., 2013). Exaggerated risk taking is also characteristic of behavioral addictions, such as pathological gambling (King et al., 2020), as well as attention-deficit hyperactivity disorder (Dekkers et al., 2016, 2022; Pollak et al., 2019). In contrast, other neuropsychiatric diseases, such as eating disorders and anxiety disorders, are associated with increased risk aversion. Individuals with these conditions undervalue potential rewarding outcomes, but overweight the potential for adverse consequences, leading to increased risk avoidance (Bernardoni et al., 2020; Lorian & Grisham, 2011). To begin to understand how risk-based decision making is altered across the spectrum of

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clinical conditions, it is necessary to identify the cognitive and neurobiological mechanisms that underlie this form of decision making under non-pathological conditions.

The use of rodent models of risk-based decision making provides opportunities to examine the neurobehavioral substrates of risk taking. The Risky Decision-making Task (RDT) is one such rodent model that recapitulates aspects of "real-world" risk taking, as it involves choices between options that differ in their relative rewards and risks of adverse consequences (Orsini et al., 2019; Simon et al., 2009). In this task, rats make discrete choices between a small, "safe" food reward and a larger, "risky" food reward that is accompanied by a varying probability of mild foot-shock punishment. On average, rats will decrease their choice of the large, risky reward as the risk of punishment increases across blocks of the task. There are, however, individual differences in risk preference, with a proportion of rats displaying preference for the large, risky reward and a separate proportion displaying preference for the small, safe reward (Orsini et al., 2020; Simon et al., 2009). Notably, these individual differences are unrelated to differences in food motivation, shock reactivity, pain sensitivity, or anxiety (Simon et al., 2011). Of particular translational relevance, individual differences in risk taking on the RDT predict aspects of cocaine self-administration behavior. For example, greater risk taking predicts greater cocaine intake during self-administration (Mitchell et al., 2014; Orsini et al., 2020). Beyond associations with drug-seeking behavior, individual differences in risk taking correlate with cognitive capabilities in other measures of executive function, such as cognitive flexibility and impulsivity. Specifically, greater choice of the large, risky reward on the RDT is associated with reduced cognitive flexibility (Shimp et al., 2015) and greater impulsive action (Gabriel et al., 2019). Such relationships are consistent with findings from studies in humans, which have shown that better working memory is associated with more adaptive risk-taking strategies (Blair et al., 2018) and that poor executive function is correlated with riskier sexual and drug-related behavior (Piche et al., 2018; Reynolds et al., 2019). Because rodent behavior on the RDT closely models many aspects of human risk-taking behavior, it is an ideal model of risk taking with which to examine the neurobehavioral mechanisms that mediate risk taking and how individual

differences in such mechanisms may contribute to vulnerability to neuropsychiatric disorders.

Despite the progress in establishing a translationally relevant rodent model of risk-based decision making and identifying its cognitive correlates, this work has been conducted almost exclusively in male subjects. This is a considerable limitation as there are significant sex differences in risk-based decision making (Islas-Preciado et al., 2020; Liley et al., 2019; Orsini et al., 2016; van den Bos et al., 2012). Indeed, corroborating observations in humans (van den Bos et al., 2013), male rats exhibit greater risk-taking behavior on the RDT compared with female rats (Orsini et al., 2016, 2020). Despite overall greater risk aversion in females, there are also individual differences in risk preference in female rats on the RDT with a similar distribution to that observed in males (Orsini et al., 2020). Like males, these individual differences in risk taking predict cocaine self-administration behavior, with greater risk preference predicting greater intake of cocaine (Orsini et al., 2020). In contrast to what is known about male risk-taking behavior, however, little is known about the cognitive or affective mechanisms that underlie risk-taking behavior in females. Such a lack of information precludes the ability to fully understand how individual differences in risk taking may confer risk and increase vulnerability to the development of neuropsychiatric diseases, particularly for those in which there are well-established sex differences in their prevalence and manifestation. For example, there is a greater incidence of generalized anxiety disorder and eating disorders, both of which are associated with extreme risk aversion, in women relative to men (McLean et al., 2011; Udo & Grilo, 2018). Hence, it is necessary to extend the characterization of the cognitive correlates of risk taking to females to gain a more comprehensive understanding of the cognitive endophenotypes that confer vulnerability to the development of neuropsychiatric diseases.

The current study was designed to investigate the cognitive mechanisms underlying risk taking in both male and female rats. Rats were behaviorally characterized on the RDT, after which they underwent a battery of behavioral tasks to evaluate various cognitive functions, including impulsive choice, working memory capacity, and cognitive flexibility (see Fig. 1 for experimental timeline). In addition to assessing sex differences in each assay, performance on
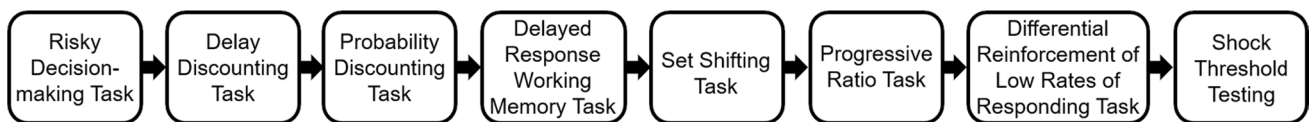
Fig. 1 *Experimental Timeline*. Male (*n* = 13) and female (*n* = 11) Long-Evans rats were behaviorally characterized on the Risky Decision-making Task until they exhibited stable choice performance. Rats were then tested on a series of behavioral tasks to assess the relationship between risk taking and measures of executive function and reward motivation

each task was correlated with risk-taking preference in the RDT. The results of this study expand upon previous work that established sex differences in risk taking and provide a more comprehensive understanding of the cognitive mechanisms that associate with risk taking in males and females.

Materials and methods

Subjects

Male ($n = 15$) and female ($n = 12$) Long-Evans rats (Charles River, Laboratories, Hollister, CA, $n = 7$ male, $n = 6$ female; Envigo, Haslett, Michigan, $n = 8$ male, $n = 6$ female; postnatal day 70 upon arrival) were individually housed in ventilated cages with Sani-Chip bedding and maintained on a 12-h reverse light/dark cycle (lights off at 0800). Two separate cohorts of rats were used (cohort 1, $n = 18$; cohort 2, $n = 9$), but the data from both cohorts were combined for analysis. Prior to experimental procedures, rats were handled daily for a minimum of 3 days to acclimate the rats to the experimenters. Rats were food restricted to 85% of their free-feeding weight, while accounting for growth (5 g increase per week) until fully grown (~250 g for females, ~350 g for males), at which point rats were fed 10 g of food per day. Rats were fed soy-free food (Envigo Teklad Irradiated Global 19% Protein Extruded Rodent Diet, #2919). All rats had ad libitum access to water and a Nyla bone for enrichment in their home cage. All procedures were conducted in accordance with The University of Texas at Austin Institutional Animal Care and Use Committee and adhered to National Institutes of Health ethical guidelines.

Apparatus

Behavioral testing was conducted in nine identical standard operant chambers (Coulbourn Instruments, Whitehall, PA). Each chamber was housed within a sound-attenuating cabinet (Coulbourn Instruments) that was outfitted with red lights and noise insulation foam. Each operant chamber contained a food trough that was located above the floor in the middle of the front wall of the chamber. Food rewards (soy-based rodent tablets; 45 mg, Lab Supply, 5UTL, Northlake, TX) were delivered into the food trough via a food hopper mounted on the back of the front wall of the chamber. Each food trough consisted of a 1.12 W light bulb to illuminate the trough during specific phases of the task as well as photobeams to register nosepoke trough entries. Two retractable levers were positioned on either side of the food trough and another 1.12 W light bulb was mounted on the back wall of the cabinet, serving as a house light. The floors of the chambers consisted of stainless-steel rods through which scrambled footshocks were delivered via their connection to

a shock generator (Coulbourn Instruments). Mounted to the ceiling of the chamber, a sensor was used to monitor locomotor activity by detecting changes in infrared (body heat) energy throughout the entire chamber. For testing in the Set-shifting Task only, two white LED cue lights were inserted into the chamber and positioned directly over each lever. Operant chambers were interfaced with a computer running Graphic State 4.0 software (Coulbourn Instruments), which concurrently controlled task events and collected behavioral data.

Behavioral procedures

Shaping

Rats were first shaped to perform separate components of the Risky Decision-making Task (RDT), including nosepoking and lever pressing. In the first phase of shaping, rats learned to nosepoke into the food trough to retrieve a single food pellet that was delivered every 100 ± 40 s. Rats were required to nosepoke into the trough at least 100 times during the 64-min session to progress to the next phase of shaping. Upon meeting this criterion, rats progressed to lever shaping wherein one lever (left or right, counterbalanced across rats) was extended into the chamber for the entire 30-min session (the other lever remained retracted for the duration of the session). A lever press would result in the delivery of one food pellet. Rats had to press the lever at least 50 times in one session to meet criterion. In the next shaping phase, rats learned to lever press the opposite lever and were required to meet the same passing criterion. In the final stage of shaping (nosepoke shaping), rats had to nosepoke into the food trough to initiate extension of the left or right lever. A press on the extended lever caused the lever to retract and the houselight to extinguish and resulted in the delivery of a single food pellet. Rats were required to lever press 30 times on each lever within a 60-min session before progressing to the next phase of training.

Reward discrimination

Before training on the RDT, rats were trained on a reward discrimination (RD) protocol, in which they learned to discriminate between a small food reward lever (one food pellet) and a large food reward lever (two food pellets). Sessions were 60 min in duration and consisted of five blocks of 18 trials. Each 40-s trial began with the illumination of the house and food trough lights. A nosepoke into the food trough extinguished the food trough light and triggered the extension of either a single lever (forced choice trials) or both levers (free choice trials) into the chamber. If rats failed to nosepoke (i.e., initiate a trial) within 10 s, lights were extinguished and the trial was scored as an omission. A press on one lever resulted

in the delivery of the small food reward and a press on the other lever resulted the delivery of the large food reward. The identity of each lever (small vs. large) was counterbalanced across males and females and remained consistent during training on the RD as well as during training on the RDT. If rats did not lever press within 10 s of lever extension, levers were retracted and the trial was scored as an omission. Following a lever press, the food trough was illuminated and levers were retracted for the remainder of the trial. The food trough light was extinguished upon collection of the food pellet or after 10 s elapsed, whichever occurred first. Each block of 18 trials began with eight forced choice trials, in which a single lever was extended (four trials for each lever, randomized across the eight trials), and ended with 10 free choice trials, in which both levers were extended and rats could choose freely between them. In contrast to the structure of the RDT (see below), all five blocks were identical in design, the intent being to teach rats the overall structure of the choice task before introducing the element of risk (footshock). Rats were trained on RD until each rat displayed a preference for the large lever ($\geq 80\%$) for 3 consecutive days, after which they progressed to the RDT. On average, rats never required more than 5 sessions to meet these criteria.

Risky Decision-making Task

The structure of the Risky Decision-making Task (RDT) was identical to that of the RD, except that the delivery of the large reward was associated with a probability of a 1-s footshock delivery. The probability of footshock systematically increased in 25% increments across the five blocks of trials, beginning at 0% and ending at 100%. Forced choice trials preceding the free choice trials were used to inform rats of the risk contingency in effect for that trial block. In these forced choice trials, the probability of footshock delivery following a lever press on the large, "risky" lever was dependent across the four trials. For example, in the 25% forced choice trial block, only one of the four forced choice trials resulted in footshock delivery. In contrast, in the 75% forced choice trial block, three of the four forced choice trials resulted in footshock delivery. Similar to RD, 10 free choice trials followed the forced choice trials. Unlike the forced choice trials, the probability of footshock delivery on an individual trial was independent of the outcome of other free choice trials in that block. Hence, the probability of footshock delivery was equivalent across all 10 trials in each block, irrespective of the outcomes of previous trials in that block. Despite the varying probability of footshock, two food pellets were always delivered when the large risky lever was pressed. Previous work shows that when trained on the RDT at the same shock intensity, males prefer the large, risky reward to a greater extent than females (Orsini et al., 2016). Consequently, males and females were trained on separate shock intensities to maximize the range of individual differences in risk taking within each sex. Shock intensities were initially set at 0.25 mA and 0.15 mA for males and females, respectively, but were adjusted over the course of training (for each sex separately) until there was a wide distribution of individual differences in risk taking. When behavioral stability emerged (see below for a definition of stability), the shock intensity for males was 0.25 mA and the shock intensities for females were 0.25 mA (cohort 1) and 0.175 mA (cohort 2).

Delay Discounting Task

The Delay Discounting Task is a well-established behavioral assay used to measure impulsive choice (Cardinal et al., 2001; Evenden & Ryan, 1996; Mendez et al., 2010; Orsini et al., 2017; Shimp et al., 2015). To ensure previous lever associations did not influence learning in the Delay Discounting Task, rats were once again trained in the nosepoke shaping protocol for a minimum of 4 days until they pressed each lever equivalently for 2 consecutive days before progressing to the Delay Discounting Task. The structure of the Delay Discounting Task (five-block design with forced and free choice trials) was similar to that of the RDT, and therefore no additional training was required prior to beginning this task. Each 60-min session consisted of five blocks of 12 trials that were 60 s in duration. Similar to RD and the RDT, each trial commenced with the illumination of the house and food trough lights. A nosepoke into the food trough triggered the extension of either a single lever (forced choice trial) or both levers (free choice levers) and extinguished the food trough light. On trials in which rats failed to nosepoke within 10 s, lights were extinguished and the trial was scored as an omission. A lever press on one lever (counterbalanced across sexes) led to the immediate delivery of one food pellet (small, immediate reward) whereas a lever press on the other lever resulted in the delivery of two food pellets after a variable delay (large, delayed reward). Failure to press a lever within 10 s led to the retraction of the lever and the trial was scored as an omission. After a lever press, the food trough was illuminated and levers were retracted for the remainder of the trial. The food trough light was extinguished after the food reward was collected or 10 s had elapsed, whichever occurred first. Each of the five trial blocks began with two forced choice trials followed by 10 free choice trials. Like the RDT, the forced choice trials were used to remind the rats of the delays associated with the large reward for that block of trials. Although remaining constant within each trial block, the delay duration between lever press and food delivery increased across the five blocks (0, 4, 8, 16, 32 s). Rats were trained on the Delay Discounting Task until stable behavior was obtained.

Probability Discounting Task

Established by St. Onge and Floresco (2009), the Probability Discounting Task is another risk-based decision-making task in which the risk associated with the large reward is that of reward omission. Before training on the Probability Discounting task, rats again underwent remedial nosepoke shaping for minimum of 4 days until they pressed each lever comparably for 2 consecutive days. Training on the Probability Discounting Task was conducted in the same chambers used for the RDT and the Delay Discounting Task. The Probability Discounting Task had a structure similar to that of the RDT and the Delay Discounting Task (five-block design with forced and free choice trials); therefore, no additional training was required. Each session was 60 min in duration and consisted of five blocks of 18 trials, each of which was 40 s in length. Trials began with the illumination of the house and food trough lights, and a nosepoke into the food trough extinguished the food trough light and prompted the extension of either a single lever (forced choice trial) or both levers (free choice trials). If a rat failed to nosepoke within 10 s, all chamber lights were extinguished and the trial was scored as an omission. A press on one lever (counterbalanced across sexes) resulted in the guaranteed delivery of a small food reward (one food pellet), whereas a press on the other lever resulted in the delivery of a large food reward (two food pellets), the probability of which systematically decreased across the five blocks of trials (100%, 50%, 25%, 12.5%, 0%). On trials in which rats failed to press a lever within 10 s, levers were retracted and the trial was scored as an omission. After a successful lever press, the food trough light was illuminated and levers were retracted. After the food reward was collected (or 10 s elapsed, whichever occurred first), the food trough light was extinguished for the remainder of the trial. Like the RDT, each block of 18 trials began with eight forced choice trials, during which the rats learned the reward probability in effect for that block, and ended with 10 free choice trials. Rats were trained on the Probability Discounting Task until behavioral stability was achieved.

Delayed Response Working Memory Task

The Delayed Response Working Memory Task is a behavioral assay used to measure the ability to retain recently acquired information "in mind" for a short period of time (Banuelos et al., 2014; Hernandez et al., 2017). Rats were trained on this task in the same operant chambers used for the decision-making tasks. Rats first underwent a lever shaping protocol in which a single lever was extended (left or right; randomized across pairs of trials) into the chamber, and a press on the lever led to the immediate delivery of one food pellet. To progress to the Delayed Response

Working Memory Task, rats were required to press each lever 30 times within a 60-min session for 3 consecutive days. Adapted from the task used by Sloan et al. (2006), the Delayed Response Working Memory Task consisted of multiple trials (the number of trials varies across rats as the task is self-paced) within a 40-min test session. The house light was illuminated throughout each test session, with the exception of timeout periods following "incorrect" responses. Each trial began with the extension of a single lever (the "sample" lever), the position of which in the chamber (left or right) was randomized within pairs of trials. A press on the lever led to its retraction and the onset of a "delay" phase. To discourage rats from sitting in front of the sample lever (and thus minimizing the use of their working memory to select the correct lever in the subsequent "choice" phase), rats were required to nosepoke in the food trough during the delay phase. The first nosepoke into the food trough detected after the delay phase elapsed triggered the extension of both levers into the chamber ("choice" phase). To receive a food reward (one food pellet), rats were required to press the same lever that had been extended in the "sample" phase (the "correct" lever). If rats pressed the opposite lever (the "incorrect" lever), no food reward was delivered, levers were retracted, and all lights in the chamber were extinguished. A 5-s intertrial interval followed a lever response, after which the next trial commenced.

In the initial sessions of the Delayed Response Working Memory Task, the delay between the sample and choice phase was set to 0 s, and correction trials were used to facilitate learning. In these trials, rats were forced to repeat a trial (beginning with the sample phase) if they pressed the "incorrect" lever on the prior trial. When rats attained greater than 80% accuracy for 2 consecutive days, they progressed through two sets of delays (delay set 1: 0, 1, 2, 3, 4, 5, 6 s; delay set 2: 0, 2, 4, 8, 12, 16 s) before reaching the final delay set (0, 2, 4, 8, 12, 18, 24 s). Rats had to achieve greater than 80% accuracy on each delay set for 2 consecutive days before proceeding to the next delay set. Rats were trained on the final delay set until stable behavior was obtained. Data from testing on the last set of delays were included in the final data analyses.

Set-shifting Task

The Set-shifting Task assesses the ability to quickly adapt to changing contingencies in the environment and is therefore considered a measure of cognitive flexibility (Floresco et al., 2008). The task design is based on that used by Floresco et al. (2008).

Side bias evaluation In the same operant boxes used for the decision-making and working memory tasks, rats were first

re-trained on the lever shaping protocol that was used prior to starting the Delayed Response Working Memory Task. To progress to the first phase of the Set-shifting Task, rats were required to press each lever 30 times within a 60-min session for 2 consecutive days. Upon reaching these criteria, rats were tested for their lever side bias. During this session, rats were presented with both levers, and a lever press on either resulted in the delivery of a single food pellet. To be rewarded on the subsequent trial, however, rats had to press the opposite lever ("correct" response). If the rat pressed the same lever as in the previous trial ("incorrect" response), the food reward was not delivered and the levers were retracted. Rats were forced to repeat the trial until the correct lever was pressed, which led to the delivery of the food reward. A rat's side bias reflected the position of the lever in the operant chamber on which the rat made the greatest number of lever presses during the entire test session.

Initial discrimination Twenty-four hours after side bias evaluation, rats began the first discrimination training wherein rats learned to discriminate between "correct" and "incorrect" levers based on the illumination of a cue light above the "correct" lever. At the beginning of each 20-s trial, a cue light was illuminated over the left or right lever (the position was randomized across pairs of trials) for 3 s, after which both levers were extended into the operant chamber for 4 s (the cue light remained on during lever extension). A "correct" response (a press on the lever below the cue light) was rewarded with a single food pellet and triggered the retraction of both levers and the termination of cue light illumination. In contrast, an "incorrect" response (a press on the lever on the side opposite to that of the illuminated cue light) did not result in food delivery and led to the retraction of the levers and termination of cue light illumination. Rats were required to complete a minimum of 30 trials out of a maximum of 120 trials in a session and to reach a criterion of eight consecutive correct responses. If these criteria were not met in a single session, rats continued training in additional initial discrimination sessions until criteria was achieved. After reaching criteria on the initial discrimination, rats received one additional discrimination session of 120 trials to reinforce the formation of the attentional "set," or the rules used to guide behavior.

Set-shift discrimination Twenty-four hours after the rats completed the initial discrimination training, rats were tested in another discrimination task in which a "set shift" occurred. The task structure (i.e., presentation of trials, etc.) was identical to that of the initial discrimination task; in this discrimination session, however, the contingencies for making a "correct" response were no longer dependent on the location of the cue light, but were instead based on the position of the lever in the chamber. Specifically, rats were

required to ignore the cue light and only respond based on the left or right position of the lever to obtain a food reward. Although the "correct" lever remained the same throughout the test session for all rats, the left/right position differed for each rat, as it was always assigned to the lever on the individual rat's "unbiased" side (based on side bias evaluation). Each session consisted of a maximum of 120 trials, and rats were trained on the set shift until they achieved a criterion of eight consecutive correct trials.

Progressive Ratio Schedule of Reinforcement Task

The progressive ratio schedule of reinforcement task (PR) has been used previously to assess rats' motivation to work for a food reward (Blaes et al., 2022; Hernandez et al., 2017; Kheramin et al., 2005; Mendez et al., 2009; Orsini et al., 2021). Testing on the PR task occurred in the same operant chambers used for the decision making, working memory and set-shifting tasks. In this task, a lever was extended into the chamber and remained extended for the duration of the session. Rats were first trained to press the lever (counterbalanced across sexes) on a fixed ratio 1 (FR1) schedule until they made 100 or more responses in a 30-min session for 2 consecutive days. On this schedule, a single lever response resulted in the illumination of the trough light and the delivery of one food pellet. Upon reaching passing criteria on the FR1 schedule, rats were tested on the PR task for 7 days. At the beginning of the session, a single lever press resulted in the delivery of one food pellet, but as the session continued, the number of presses required to obtain a food reward increased in an arithmetic progression based on the following sequence: 1, 3, 6, 10, 15, 21, 28, 36, 45, 55, etc. The session finished when 10 min had elapsed since the last reward was delivered.

Differential Reinforcement of Low Rates of Responding Task

The Differential Reinforcement of Low Rates of Responding Task (DRL) has previously been used as a way to measure impulsive action in rodents (Gabriel et al., 2019; Hankosky & Gulley, 2013). In the same operant boxes used for the previous behavioral assays, rats were re-trained on a lever shaping protocol in which one of the two levers (left or right, presented in a randomized order) was inserted into the chamber. A single lever press resulted in delivery of a single food pellet and retraction of the lever. Rats were trained on this protocol for 4 days, after which they began training on the DRL to assess impulsive action. On the DRL, rats had to press a lever twice to obtain one food pellet. After the first lever press, however, rats were required to withhold the second lever press for a predetermined delay period. If a second lever press was made before the delay period elapsed, the delay period restarted. If the rat did not initiate a trial within 10 s, the lever

was retracted and the house light was extinguished for two seconds. The initial delay period was set at 5 s, and rats were trained on the DRL with this delay until they reached passing criteria of 80% "correct" responses (i.e., responses that were reinforced) for 2 consecutive days. Upon reaching criteria, rats advanced to the next delay (10 s, 20 s). In addition to tracking the number of trials to reach criteria and the number of reinforced lever presses, behavior was also quantified by calculating the ratio of correct responses (at each delay) to the total number of responses made in the entire session.

Determination of shock reactivity thresholds

At the completion of all behavioral testing, rats underwent shock threshold testing to identify the lowest shock intensity that would elicit a motor response to the footshock. After a 1-min acclimation period, an initial 1-s 0.4 mA footshock was delivered to decrease spontaneous motor activity and enable observations of paw flinches at lower shock intensities. A series of shocks were subsequently delivered at 10-s intervals, beginning at 0.05 mA. Shock intensities were increased by 0.025 mA until a paw flinch was elicited, at which point the shock intensity was decreased by 0.025 mA. Trials continued in this "up-and-down" manner (Crocker & Russell, 1984) until five flinch responses were observed, with the last followed by a trial on which no flinch was observed. Shock intensities were averaged to obtain a mean shock reactivity threshold for each rat.

Data analyses

Power analyses were conducted a priori to determine appropriate sample sizes to detect effect sizes of 0.8 or greater, assuming an α of 0.05. Data were extracted and analyzed using customized Graphic State 4.0 analysis templates for each behavioral task. Statistical analyses were conducted with SPSS 27.0 and figures were created with GraphPad Prism 9.0. Because rats were sourced from two different commercial suppliers, vendor was entered as a covariate in each analysis. If the analysis revealed that vendor was a significant covariate, it was included as a covariate in the analysis of the dependent variable of interest using an ANCOVA and presented in the results. If vendor was not identified as a significant covariate, analyses were conducted without vendor as a covariate using an ANOVA or independent samples t-test. Results were considered statistically significant when $p \leq 0.05$. If parent ANOVAs (or ANCOVAs) yielded main effects or significant interactions, additional post-hoc ANOVAs (or ANCOVAs) were conducted to identify the source of the significance, and Bonferroni-adjusted p-values were used to determine if the results were statistically significant. Finally, effect sizes are reported as η^2 for ANOVAs and the absolute value of Cohen's d for independent sample's t-tests.

Risky Decision-making Task

The primary dependent variable was the percentage of free choice trials in each block on which a rat chose the large, risky reward (risk taking). To determine behavioral stability, a repeated-measures analysis of variance (ANOVA) was used to analyze risk taking across a sliding window of three consecutive sessions, with day and trial block (0, 25, 50, 75, 100%) as within-subjects factors and sex as the between-subjects factor. Stable behavior was achieved when there was a main effect of trial block, but no main effect of day nor a day X trial block interaction. Sex differences in risk taking in the RDT were assessed with a two-factor repeated-measures ANCOVA, with trial block (averaged across the 3 days of stable behavior) as the within-subjects factor, sex as the between-subjects factor and vendor as a significant covariate. To determine if sex differences in risk taking were due to differences in the extent to which the outcome of the previous trial (large, punished outcome vs. large, unpunished outcome) affected choice in the next trial (choice of the safe vs. risky lever), trial-by-trial analyses were conducted on stable choice behavior. Win-stay behavior, which provided a measure of reward sensitivity, was calculated by dividing the number of free choice trials on which a rat chose the large, risky lever after receiving the large, unpunished reward by the total number of free choice trials on which the rat received the large, unpunished reward. Conversely, lose-shift behavior, which provided a measure of sensitivity to punishment, was calculated by dividing the number of free choice trials on which a rat chose the small, safe lever after receiving the large, punished reward by the total number of free choice trials on which the rat received the large, punished reward. Once calculated for each rat, these variables were analyzed with independent sample's t-tests, with sex as the between-subjects factor. For use in subsequent correlational analyses, percent choice of the large, risky reward was averaged across trial blocks 2–5 (blocks of trials in which risk of punishment was present) of stable behavior. Because vendor was a significant covariate in the analysis of sex differences in risk taking, partial correlations were used when examining relationships between risk taking and measures in other behavioral tasks. As an additional means of assessing the relationship between risk taking and other cognitive measures, rats were split into "risk-taking" and "risk-averse" groups by conducting a median split of percent choice of the large, risky reward averaged across blocks 2–5 (separately for males and females) of stable performance on the RDT. Performance on other behavioral tasks was then compared between these two groups (separately for males and females) using a repeated-measures ANOVA or an independent samples t-test, with behavioral phenotype (risk-taking vs. risk-averse) as the between-subjects factor.

Latency to press levers in forced choice trials was also evaluated with a repeated-measures ANCOVA with lever identity (small, safe vs large, risky) and trial block as within-subjects factors, sex as the between-subjects factor and vendor as a significant covariate. Latency to press levers during the free choice trials was not analyzed as there was frequently insufficient data for trial blocks in which rats chose one lever exclusively (i.e., only the small lever or only the large lever). Further, in contrast to latency to press levers during free choice trials, latency to press levers during forced choice trials provides a measure of incentive motivation that is dissociated from processes related to decision making or evaluation of relative reward magnitudes. Other ancillary behavioral measures were analyzed, including baseline locomotor activity, locomotor activity during shock delivery and percentage of omitted free choice trials. Baseline locomotor activity (i.e., activity during the intertrial intervals) was averaged across the five blocks of trials and across the 3 days of stable behavior. Locomotor activity during shock delivery (1 s) was calculated similarly, except that only the blocks in which shocks occurred (trial blocks 2–5) were included. Omissions were determined by dividing the number of omitted free choice trials by the total number of trials in a block. These values were then averaged across the 3 days of stable behavior. Baseline locomotor activity was analyzed with an ANCOVA, with sex as the between-subjects factor and vendor as a significant covariate. Locomotor activity during

shock delivery and omissions were both analyzed using an independent samples t-test, with sex as the between-subjects factor. Descriptive statistics for locomotor activity and omissions for all behavioral assays are presented in Table 1 and statistical results for these measures are presented in Table 2.

Delay Discounting Task

For the Delay Discounting Task, the primary dependent variable was the percentage of free choice trials in each block on which a rat chose the large, delayed reward. Behavioral stability was assessed with identical analyses to those used to determine stability on the RDT. Sex differences in choice of the large, delayed reward were evaluated with a two-factor repeated-measures ANOVA, with trial block (averaged across the 3 days of stable behavior) as the within-subjects factor and sex as the between-subjects factor. To examine correlations between impulsive choice (i.e., performance on the Delay Discounting Task) and risk taking, percent choice of the large, delayed reward during stable performance on the Delay Discounting Task was averaged across the blocks in which there was a delay (blocks 2–5) and this value was used in subsequent correlational analyses (see above for details on the type of correlational analysis used; separately for each sex). Additionally, impulsive choice was compared between risk-taking and risk-averse rats using

Table 1 Mean (± standard error of the mean) locomotor activity and omissions

	Locomotor activity (locomotor units/ITI)	Shock reactivity (locomotor units/shock)	Omissions (% of trials)
Risky Decision-making Task			
Male	24.12 (3.04)	4.98 (0.924)	0.31 (0.122)*
Female	17.75 (3.45)	3.84 (0.875)	7.88 (2.15)*
Delay Discounting Task			
Male	37.82 (3.00)*	N/A	1.08 (0.862)*
Female	25.90 (5.76)*	N/A	8.18 (2.71)*
Probability Discounting Task			
Male	27.29 (2.54)*	N/A	0.256 (0.142)*
Female	16.00 (3.51)*	N/A	8.30 (2.85)*
Delayed Response Working Memory Task			
Male	21.01 (2.09)	N/A	N/A
Female	19.22 (3.19)	N/A	N/A
Set-shifting Task			
Initial discrimination			
Male	21.92 (1.89)	N/A	N/A
Female	18.13 (2.78)	N/A	N/A
Set shift			
Male	22.83 (1.69)	N/A	N/A
Female	17.53 (2.94)	N/A	N/A

*Indicates main effect of sex

Table 2 Sex and vendor differences in ancillary behavioral measures on the Risky Decision-making Task and other behavioral tasks

	Factor(s)	F- or t-value	p-value	Effect size (η2 or d)	Vendor/Sex Difference
Risky Decision-making Task					
Locomotor activity (locomotor units/ITI)	Sex#	F (1,21) = 2.66	0.12	0.11	
	Vendor	F (1,21) = 6.07	0.02*	0.22	CR > ENV
Shock reactivity (locomotor units/shock)	Sex	t (21) = 0.87	0.39	0.37	
Omissions (% of trials)	Sex	t (22) = -3.84	< 0.05*	1.57	M < F
Delay Discounting Task					
Locomotor activity (locomotor units/ITI)	Sex#	F (1,21) = 4.46	0.05*	0.18	M > F
	Vendor	F (1,21) = 5.25	0.03*	0.20	CR > ENV
Omissions (% of trials)	Sex	t (22) = -2.68	0.01*	1.10	M < F
Probability Discounting Task					
Locomotor activity (locomotor units/ITI)	Sex#	F (1,21) = 8.77	< 0.01*	0.30	M > F
	Vendor	F (1,21) = 5.89	0.02*	0.22	CR > ENV
Omissions (% of trials)	Sex#	F (1,21) = 11.95	< 0.01*	0.36	M < F
	Vendor	F (1,21) = 6.53	0.02*	0.24	CR < ENV
Delayed Response Working Memory Task					
Locomotor activity (locomotor units/ITI)	Sex	t (22) = 0.48	0.63	0.20	
Set-shifting Task					
Initial discrimination: Locomotor activity (locomotor units/ITI)	Sex#	t (22) = 1.16	0.26	0.47	
	Vendor	F (1,21) = 4.19	0.05*	0.17	CR > ENV
Set shift: Locomotor activity (locomotor units/ITI)	Sex	F (1,21) = 3.07	0.09	0.13	

Indicates $p \leq 0.05$

#*Indicates vendor was a significant covariate*

CR, Charles River; *ENV*, Envigo; *M*, Male; *F*, Female

a repeated-measures ANOVA, with trial block included as the within-subjects factor and risk-taking phenotype as the between-subjects factor.

Similar to analysis for the RDT, latency to press levers during forced choice trials was also evaluated with a repeated-measures ANOVA, with lever identity (small, immediate vs. large, delayed) and trial block as within-subjects factors, and sex as the between-subjects factor. Baseline locomotor activity (i.e., activity during the inter-trial intervals) and percentage of omitted free choice trials were calculated in a manner identical to that used to calculate these variables in the RDT. Baseline locomotor activity was analyzed using an ANCOVA, with sex as the between-subjects factor and vendor as a significant covariate. Omissions were analyzed using an independent samples *t*-test, with sex as the between-subjects factor.

Probability Discounting Task

The primary dependent variable in the Probability Discounting Task was the percentage of free choice trials in each block on which a rat chose the large, uncertain reward (risky choice). Analyses identical to those used for the RDT and the Delay Discounting Task were used to determine behavioral stability on the Probability Discounting Task. Sex differences in risky choice were assessed with a two-factor repeated-measures ANOVA, with trial block (averaged across the 3 days of stable behavior) as the within-subjects factor and sex as the between-subjects factor. To evaluate correlations between risky choice in the Probability Discounting Task and risk taking in the RDT, percent choice of the large, uncertain reward during stable performance on the Probability Discounting Task was

averaged across the trial blocks in which there was a probability of omission of the large reward (blocks 2–5) and this value was used in subsequent correlational analyses (separately for each sex). Risky choice in the Probability Discounting Task was compared between risk-taking and risk-averse rats using a repeated-measures ANOVA, with trial block as the within-subjects factor and risk-taking phenotype as the between-subjects factor.

Latency to press levers during forced choice trials was analyzed with a three-factor repeated-measures ANCOVA, with lever identity (small, certain vs. large, uncertain) and trial block as within-subjects factors, sex as the between-subjects factor and vendor as a significant covariate. Baseline locomotor activity (i.e., activity during intertrial intervals) and percentage of omitted free choice trials were calculated in a manner identical to that used to calculate these measures for the RDT and Delay Discounting Task. Both of these variables were then subjected to an ANCOVA, with sex as the between-subjects factor and vendor as a significant covariate.

Delayed Response Working Memory Task

The primary dependent variable for the Delayed Response Working Memory Task was percentage of correct trials (i.e., accuracy) at each delay. Once rats reached the third delay set, accuracy across blocks was assessed across a sliding window of 5 days until behavioral stability was attained, as determined by a repeated-measures ANOVA (main effect of delay, but neither a main effect of day nor a significant day X delay interaction). Upon reaching stability, working memory performance at each delay was averaged across the 5 days of stable performance and subjected to a repeated-measures ANOVA, with delay as the within-subjects factor and sex as the between-subjects factor. The total number of completed trials was also compared between sexes using an ANOVA, with sex as the between-subjects variable. For correlations between choice accuracy in the Delayed Response Working Memory task and risk taking, accuracy for each delay block during stable performance (i.e. 0, 2, 4, 8, 12, 18, 24 s) on the Delayed Response Working Memory Task was averaged to produce a mean accuracy value, which was then used in subsequent correlational analyses (separately for each sex). Choice accuracy was also compared between risk-taking and risk-averse rats (separately for each sex), with delay as the within-subjects factor and risk-taking phenotype as the between-subjects factor. Correlational analyses were also used to evaluate the association between risk taking in the RDT and total number of completed trials for each sex. An independent samples t-test was used to compare the total number of completed trials between risk-taking and risk-averse rats (separately for each sex).

Latencies to press levers during the choice phase of stable behavior were also analyzed with a repeated-measures ANOVA. In this analysis, levers (correct vs. incorrect) and delay were included as the within-subjects factors, while sex was included as the between-subjects factor. Finally, locomotor activity across each session was averaged across the 5 days of stable behavior and compared between sexes (between-subjects factor) using an independent samples t-test.

Set-shifting Task

Initial discrimination The number of trials to reach criterion performance and the number of errors were the primary dependent variables on the initial discrimination. An independent samples t-test was used to compare these variables between males and females. Both variables were also used in correlational analysis to assess their relationship with performance on the RDT. An independent sample's t-test was employed to compare performance on the initial discrimination between risk-taking and risk-averse rats (separately for each sex).

Set shift The number of trials to reach criterion and the number of errors made during the set shift were the primary dependent variables on the set-shift phase of the Set-shifting Task. Errors were categorized as previously reinforced (when the locations of the cue light and the lever were distinct and a choice was made based on the contingencies learned in the initial discrimination) or never reinforced (when the locations of the cue light and lever were the same and a choice was made that was not based on contingencies learned in either type of discrimination). These variables were compared between sexes using an independent samples t-test. To examine the relationship between risk taking and cognitive flexibility, the number of trials to reach criterion and the number of previously reinforced or never reinforced errors on the set shift were correlated with risk taking in the RDT (separately for each sex). Further, these variables were compared between risk-taking and risk-averse rats using an independent sample's t-test.

Locomotor activity during the initial discrimination was analyzed using an independent sample's t-test, with sex as a between-subjects factor. Because vendor was a significant covariate in the analysis of locomotor activity during the set shift, an ANCOVA was used to compare locomotor activity between males and females in this phase of the task. If subjects required more than one session to reach criteria on the set-shift, locomotor activity was averaged across the sessions and then subjected to analysis.

Progressive Ratio Schedule of Reinforcement Task

For the PR assay, the average number of lever presses, the average ratio, and the average amount of food earned across 7 days of testing were analyzed with an ANCOVA, with sex as the between-subjects factor and vendor as a significant covariate. These behavioral measures were also used in correlational analyses evaluating the relationship between motivation to work for food and risk taking in the RDT. In addition, the number of lever presses, PR ratios and food earned were compared between risk-taking phenotypes using an ANCOVA for each sex, with phenotype as the between-subjects factor and vendor as a significant covariate.

Differential Reinforcement of Low Rates of Responding Task

On the DRL task, the number of trials to reach criteria, the number of reinforced lever presses and the ratio of correct responses were each analyzed with an independent samples t-test for each DRL delay, with sex as a between-subjects factor. These variables were also used in correlational analyses (separately for each sex) to examine the relationship between impulsive action and risk taking in the RDT. Independent samples t-tests were conducted separately for each sex to compare these behavioral measures at each DRL delay between risk-taking and risk-averse rats.

Determination of shock reactivity thresholds

Shock reactivity thresholds were analyzed with an independent samples t-test, with sex as the between-subjects factor. These values were used in correlational analyses (separately for each sex) to evaluate the relationship between shock reactivity and risk taking in the RDT. Separate ANCOVAS were conducted for males and females to compare shock reactivity thresholds between risk-taking and risk-averse rats, with risk-taking phenotype as the between-subjects factor and vendor as a significant covariate.

Results

Risky Decision-making Task

Three rats ($n=2$, male; $n=1$, female) were excluded from the study due to their inability to learn the task contingencies on the RDT in a manner comparable to the other subjects in the study. Male ($n=13$) and female ($n=11$) rats were trained on the RDT for 30–45 days, at which point choice behavior was stable [day, $F(2, 42)=0.25$, $p=0.78$, $\eta^2=0.01$; trial block, $F(4, 84)=50.73$, $p<0.01$, $\eta^2=0.71$; day X trial block, $F(8, 168)=0.74$, $p=0.65$, $\eta^2=0.03$] in both sexes [sex X day, $F(2, 42)=0.21$, $p=0.82$, $\eta^2=0.01$; day X sex X trial block,

$F(8, 168)=1.01$, $p=0.43$, $\eta^2=0.05$]. Vendor was a significant covariate for performance on the RDT [$F(1, 21)=4.73$, $p=0.04$, $\eta^2=0.18$], with rats from Charles River exhibiting greater choice of the large, risky reward than rats from Envigo, and was thus included as such in the analysis of sex differences in risk taking. Analysis of performance across the 3 days of stable behavior revealed that rats decreased their choice of the large, risky reward (i.e., decreased risk taking) as the risk of punishment increased across the session [trial block, $F(4, 84)=3.01$, $p=0.02$, $\eta^2=0.13$]. There was a greater reduction in risk taking in females than males [Fig. 2A; sex, $F(1, 21)=4.79$, $p=0.04$, $\eta^2=0.19$; sex X trial block interaction, $F(4, 84)=3.21$, $p=0.02$, $\eta^2=0.13$], replicating previous work showing greater risk aversion in females relative to males (Orsini et al., 2016). Trial-by-trial analysis showed that although there were no differences between males and females on win-stay behavior [Fig. 2B; $t(21)=1.33$, $p=0.20$, $d=0.56$], females displayed greater lose-shift behavior than males [$t(22)=1.87$, $p=0.05$, $d=0.85$], consistent with their risk-averse phenotype. Despite greater risk aversion in females overall, there was a distribution of risk preferences in both males (Fig. 2C) and females (Fig. 2D).

Vendor was a significant covariate in the analyses of latency to press levers during forced choice trials [$F1, 21)=18.51$, $p<0.01$, $\eta^2=0.47$; rats from Envigo had longer latencies to press levers than rats from Charles River] and was thus included as such in the analyses of latencies to press levers. There was no main effect of lever identity [$F(1, 21)=0.12$, $p=0.73$, $\eta^2<0.01$] nor an interaction between lever identity and trial block [$F(4, 84)=0.57$, $p=0.69$, $\eta^2=0.03$]. There were, however, sex differences in latencies to press levers [sex, $F(1, 21)=29.01$, $p<0.01$, $\eta^2=0.58$; lever identity X sex, $F(1, 21)=4.29$, $p=0.05$, $\eta^2=0.17$; lever identity X sex X trial block, $F(4, 84)=8.42$, $p<0.01$, $\eta^2=0.29$]. To identify the source of these interactions, separate repeated-measures ANOVAs were conducted for each lever, comparing latencies between males and females. Females had longer latencies to press the small lever than males across all trial blocks compared with males [sex, $F(1, 21)=11.98$, $p<0.01$, $\eta^2=0.36$; trial block, $F(4, 84)=0.46$, $p=0.76$, $\eta^2=0.02$; sex X trial block, $F(4, 84)=1.15$, $p=0.34$, $\eta^2=0.05$]. Similarly, females took longer than males to press the large lever and their latencies to press this lever increased as the risk of punishment increased [sex, $F(1, 21)=15.12$, $p<0.01$, $\eta^2=0.42$; trial block, $F(4, 84)=0.77$, $p=0.55$, $\eta^2=0.04$; sex X trial block, $F(4, 84)=10.63$, $p<0.01$, $\eta^2=0.34$].

Delay Discounting Task

Performance on the Delay Discounting Task

Rats were trained on the Delay Discounting Task for 22–32 days until stable behavior emerged [day, $F(2,$
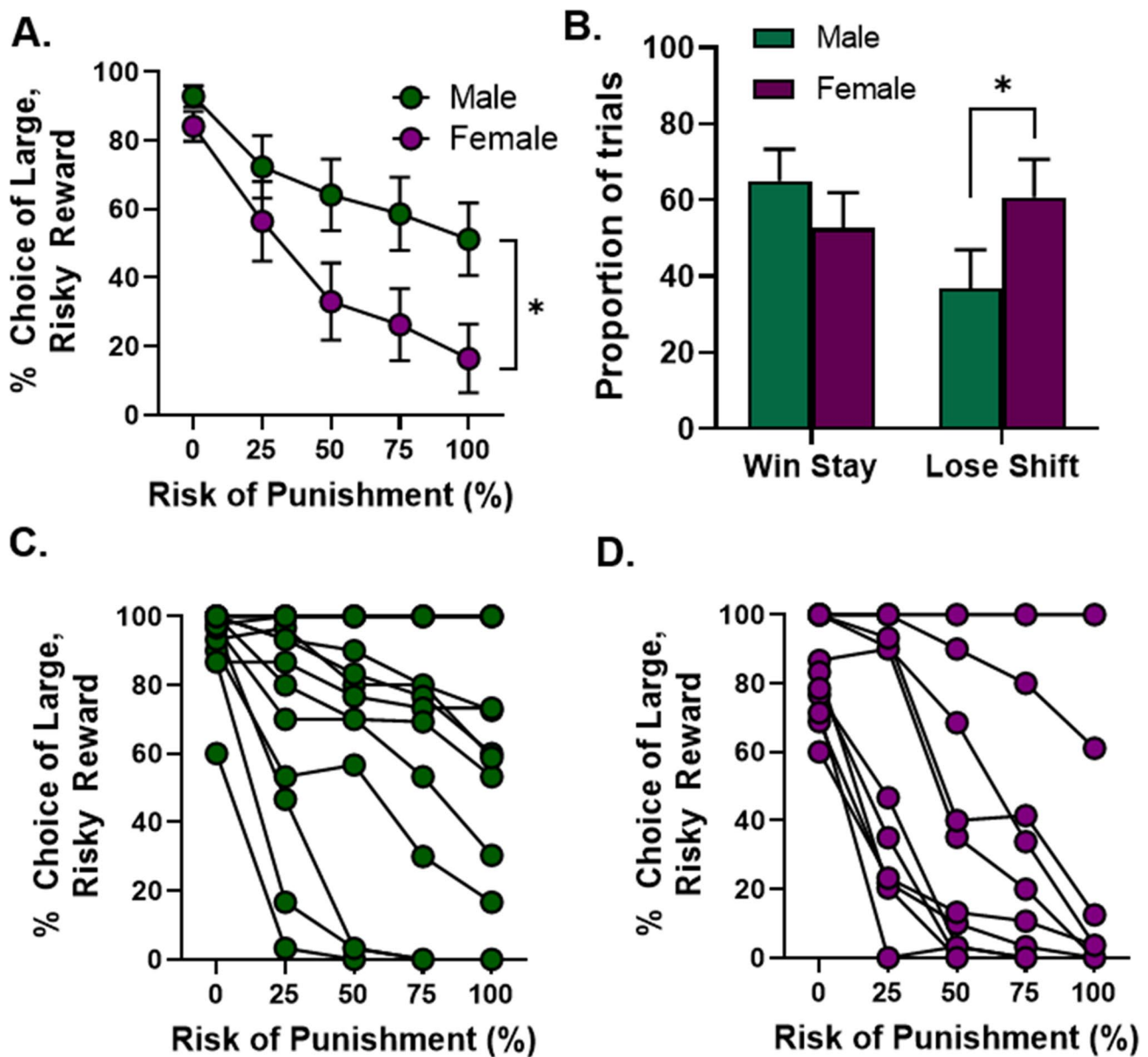
Fig. 2 *Performance on the Risky Decision-making Task in Male and Females.* **A.** Males preferred the large, risky reward significantly more than females. **B.** Females exhibited greater lose-shift behavior compared with males. **C, D.** There were individual differences in performance on the Risky Decision-making Task in males (**C**) and females (**D**). Data are represented as mean ($\pm$) standard error of the mean. Asterisks indicate $p < 0.05$

$40) = 1.18$, $p = 0.32$, $\eta^2 = 0.06$; trial block, $F (4, 80) = 154.83$, $p < 0.01$, $\eta^2 = 0.89$; day X trial block, $F (8, 160) = 0.82$, $p = 0.59$, $\eta^2 = 0.04$] in both males and females [day X sex, $F (2, 40) = 0.85$, $p = 0.44$, $\eta^2 = 0.04$; day X sex X trial block, $F (8, 160) = 0.60$, $p = 0.78$, $\eta^2 = 0.03$]. A two-factor repeated-measures ANOVA of stable behavior revealed that rats chose the large, delayed reward significantly less as the delays increased [trial block, $F (4, 88) = 152.27$, $p < 0.01$, $\eta^2 = 0.76$] and that this did not differ between males and females [Fig. 3A; sex, $F (1, 22) = 0.28$, $p = 0.60$, $\eta^2 = 0.16$;

sex X trial block, $F (4, 88) = 0.15$, $p = 0.96$, $\eta^2 = 0.12$]. Using a three-factor repeated-measures ANOVA, analyses of latency to press levers showed that rats had longer latencies to press the large lever than the small lever [$F (1, 19) = 12.64$, $p < 0.01$, $\eta^2 = 0.40$] and that these latencies increased as delays to the large reward increased [$F (4, 76) = 8.37$, $p < 0.01$, $\eta^2 = 0.31$]. This behavioral pattern did not differ between males and females [lever identity X sex, $F (1, 19) = 2.37$, $p = 0.14$, $\eta^2 = 0.11$; lever identity X sex X trial block, $F (4, 76) = 0.26$, $p = 0.90$, $\eta^2 = 0.01$], although
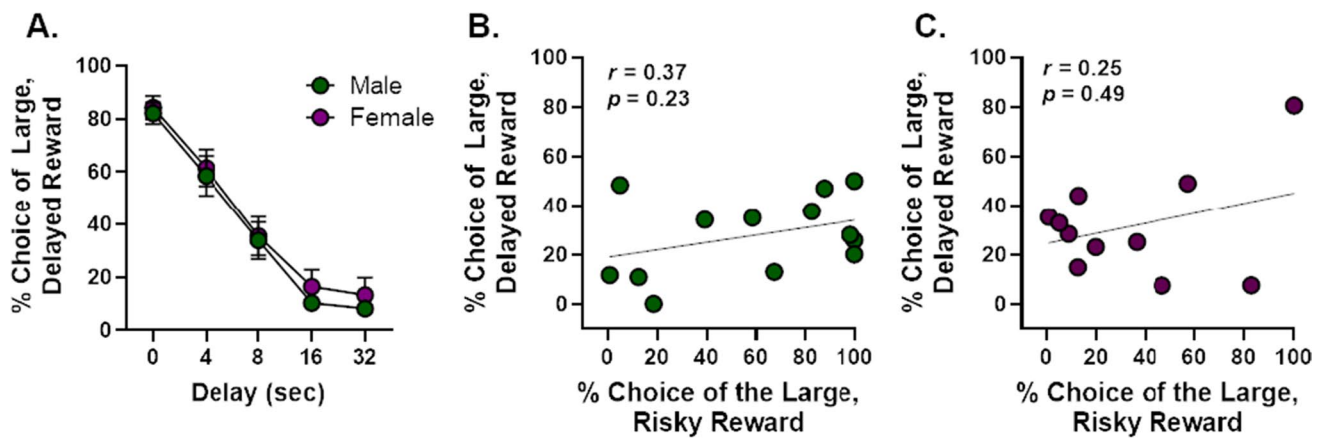
Fig. 3 *Performance on the Delay Discounting Task in Male and Females.* **A.** There were no differences between males and females in choice of the large, delayed reward. Data are represented as mean ($\pm$) standard error of the mean. **B, C.** There was no significant correlation between choice of the large, risky reward on the Risky Decision-making Task and choice of the large, delayed reward on the Delay Discounting Task in males (**B**) or females (**C**). Each data point represents the average choice of the large reward across trial blocks 2–5 on each task for each individual rat

females had longer latencies overall relative to males [F (1, 19) = 11.51, $p < 0.01$, $\eta^2 = 0.38$].

Relationship between performance on the RDT and the Delay Discounting Task

Results of all correlational analyses are presented in Table 3. Risk taking in the RDT was not significantly correlated with impulsive choice on the Delay Discounting Task in males (Fig. 3B; $r = 0.37$, $p = 0.23$) or females (Fig. 3C; $r = 0.25$, $p = 0.49$). There were also no differences in impulsive choice between risk-taking and risk-averse males [trial block, F (4, 44) = 76.97, $p < 0.01$, $\eta^2 = 0.88$; group, F (1, 11) = 0.53, $p = 0.48$, $\eta^2 = 0.05$; group X trial block, F (4, 44) = 0.47, $p = 0.76$, $\eta^2 = 0.04$] or between risk-taking and risk-averse females [trial block, F (4, 36) = 79.00, $p < 0.01$, $\eta^2 = 0.90$; group, F (1, 9) < 0.01, $p = 0.99$, $\eta^2 < 0.01$; group X trial block, F (4, 36) = 1.51, $p = 0.25$, $\eta^2 = 0.14$].

Probability Discounting Task

Performance on the Probability Discounting Task

Rats were trained on the Probability Discounting Task for 25–28 days, at which point stable behavior emerged [day, F (2, 40) = 0.17, $p = 0.85$, $\eta^2 < 0.01$; day X trial block, F (8, 160) = 1.44, $p = 0.18$, $\eta^2 = 0.07$] in both males and females [day X sex, F (2, 40) = 2.41, $p = 0.10$, $\eta^2 = 0.11$; day X sex X trial block, F (8, 160) = 1.56, $p = 0.14$, $\eta^2 = 0.07$]. Using a two-factor repeated-measures ANOVA, analysis

of stable behavior revealed no differences in choice of the large, uncertain reward between males and females [Fig. 4; trial block, F (4, 88) = 199.99, $p < 0.01$, $\eta^2 = 0.90$; sex, F (1, 22) = 0.42, $p = 0.53$, $\eta^2 = 0.02$; sex X trial block, F (4, 88) = 0.21, $p = 0.93$, $\eta^2 < 0.01$]. There were, however, sex differences in latencies to press levers during the forced choice trials, similar to those observed in the RDT. Vendor was a significant covariate in analyses of latencies [F (1, 21) = 6.09, $p = 0.02$, $\eta^2 = 0.23$] and was therefore included as such in these analyses. Although there was no main effect of lever identity [F (1, 21) = 0.07, $p = 0.79$, $\eta^2 < 0.01$] nor were there significant interactions between lever identity and sex [F (1, 21) = 2.98, $p = 0.10$, $\eta^2 = 0.02$] or lever identity and trial block [F (4, 84) = 1.90, $p = 0.12$, $\eta^2 = 0.08$], there was a main effect of sex [F (1, 21) = 20.64, $p < 0.01$, $\eta^2 = 0.50$] and a significant interaction between lever identity, sex and trial block [F (4, 84) = 7.41, $p < 0.01$, $\eta^2 = 0.26$]. These results indicate that changes in latencies to press the small versus large reward across trial blocks differed between males and females. To identify the source of these significant inter-actions, additional repeated-measures ANOVAs were conducted separately for each lever. Females took significantly longer to press the small, certain lever across all trial blocks compared with males [trial block, F (4, 84) = 2.08, $p = 0.09$, $\eta^2 = 0.09$; sex, F (1, 21) = 18.19, $p < 0.01$, $\eta^2 = 0.46$; sex X trial block, F (4, 84) = 0.23, $p = 0.92$, $\eta^2 = 0.01$]. Similarly, not only were latencies to press the large lever longer in females than males [F (1, 21) = 40.10, $p < 0.01$, $\eta^2 = 0.37$], but these latencies also increased at a greater rate across the trial blocks in females relative to males [trial block, F (4, 84) = 0.68, $p = 0.61$, $\eta^2 = 0.03$; sex X trial block, F (4, 84) = 8.59, $p < 0.01$, $\eta^2 = 0.29$].

Table 3 Summary of correlations between risk taking on the Risky Decision-making Task and dependent measures in other behavioral tasks

	Male	Female
Delay Discounting		
Percent Choice	$r=0.37$; $p=0.23$	$r=0.25$; $p=0.49$
Probability Discounting Task		
Percent Choice	$r=-0.47$; $p=0.12$	$r=-0.13$; $p=0.72$
Delayed Response Working Memory Task		
Accuracy	$r=-0.07$; $p=0.83$	$r=0.16$; $p=0.65$
Trials Completed	$r=0.27$; $p=0.40$	$r=0.35$; $p=0.33$
Set-shifting Task		
Trials to Criterion	$r=-0.17$; $p=0.59$	$r=-0.17$; $p=0.63$
Previously Reinforced Errors	$r=-0.54$; $p=0.05$	$r=-0.04$; $p=0.92$
Never Reinforced Errors	$r=-0.14$; $p=0.66$	$r=-0.33$; $p=0.30$
Progressive Ratio Schedule of Reinforcement Task		
Lever presses	$r=0.03$; $p=0.92$	$r=-0.70$; $p=0.03$*
Food earned	$r=-0.10$; $p=0.76$	$r=-0.58$; $p=0.08$#
Breakpoint	$r=-0.06$; $p=0.85$	$r=-0.67$; $p=0.03$*
Differential Reinforcement of Low Rates of Responding Task		
DRL5		
Lever presses	$r=-0.12$; $p=0.72$	$r=0.30$; $p=0.47$
Sessions to criteria	$r=-0.15$; $p=0.67$	$r=0.18$; $p=0.66$
Ratio of correct responses	$r=-0.24$; $p=0.49$	$r=0.09$; $p=0.83$
DRL10		
Lever presses	$r=-0.09$; $p=0.80$	$r=-0.08$; $p=0.86$
Sessions to criteria	$r=0.20$; $p=0.56$	$r=0.24$; $p=0.56$
Ratio of correct responses	$r=-0.27$; $p=0.42$	$r=-0.44$; $p=0.27$
DRL20		
Lever presses	$r=-0.18$; $p=0.60$	$r=-0.37$; $p=0.36$
Sessions to criteria	$r=0.09$; $p=0.80$	$r=-0.17$; $p=0.69$
Ratio of correct responses	$r=0.07$; $p=0.84$	$r=-0.38$; $p=0.35$
Shock Reactivity Thresholds		
Thresholds	$r=0.59$; $p=0.04$*	$r=0.62$; $p=0.06$#

#*Indicates a near-significant correlation*

**Indicates a significant correlation*

Vendor has been controlled for in all correlational analyses

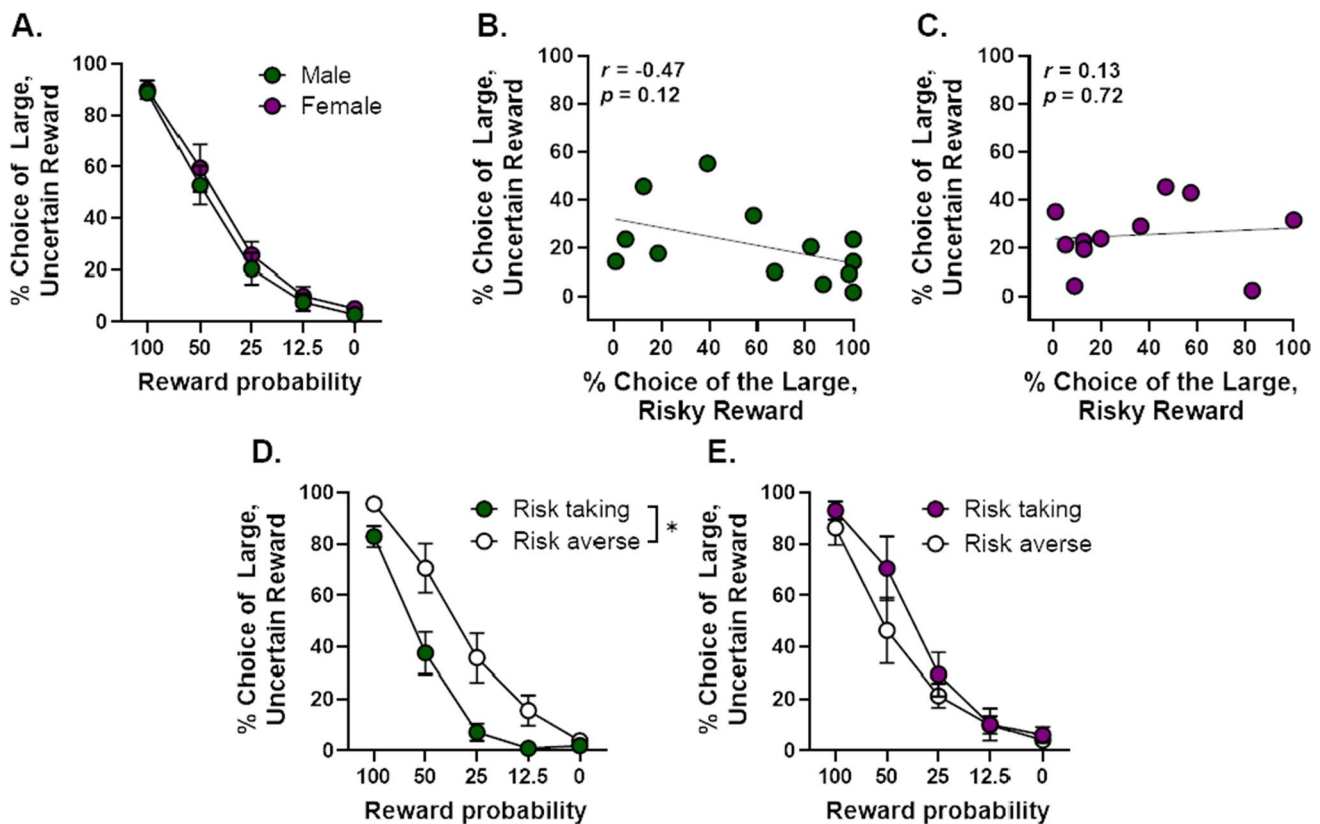### Relationship between performance on the RDT and the Probability Discounting Task

Correlational analyses revealed that risk taking in the RDT was not significantly associated with risky choice in the Probability Discounting Task in males (Fig. 4B; $r=-0.47$, $p=0.12$) or females (Fig. 4C; $r=-0.13$, $p=0.72$). There were, however, differences in choice of the large, uncertain reward between risk-taking and risk-averse male rats, with risk-taking rats choosing the large, uncertain reward significantly less than risk-averse rats [Fig. 4D; trial block, $F(4, 44)=164.49$, $p<0.01$, $\eta^2=0.94$; group, $F(1, 11)=9.45$, $p=0.01$, $\eta^2=0.46$; group X trial block, $F(4, 44)=4.96$, $p<0.01$, $\eta^2=0.31$]. These differences, however,

were not observed in female rats [Fig. 4E; trial block, $F(4, 36)=83.11$, $p<0.01$, $\eta^2=0.90$; group, $F(1, 9)=1.11$, $p=0.32$, $\eta^2=0.11$; group X trial block, $F(4, 36)=1.45$, $p=0.24$, $\eta^2=0.14$].

Delayed Response Working Memory Task

Performance on the Delayed Response Working Memory Task

Male and female rats were trained on the Delayed Response Working Memory Task for 27–43 days (12–20 days on delay sets 1 and 2; 15–23 days on final delay set) until stable behavior emerged on the final set of delays [day, $F(4, 88)=1.20$, $p=0.32$, $\eta^2=0.05$; delay,
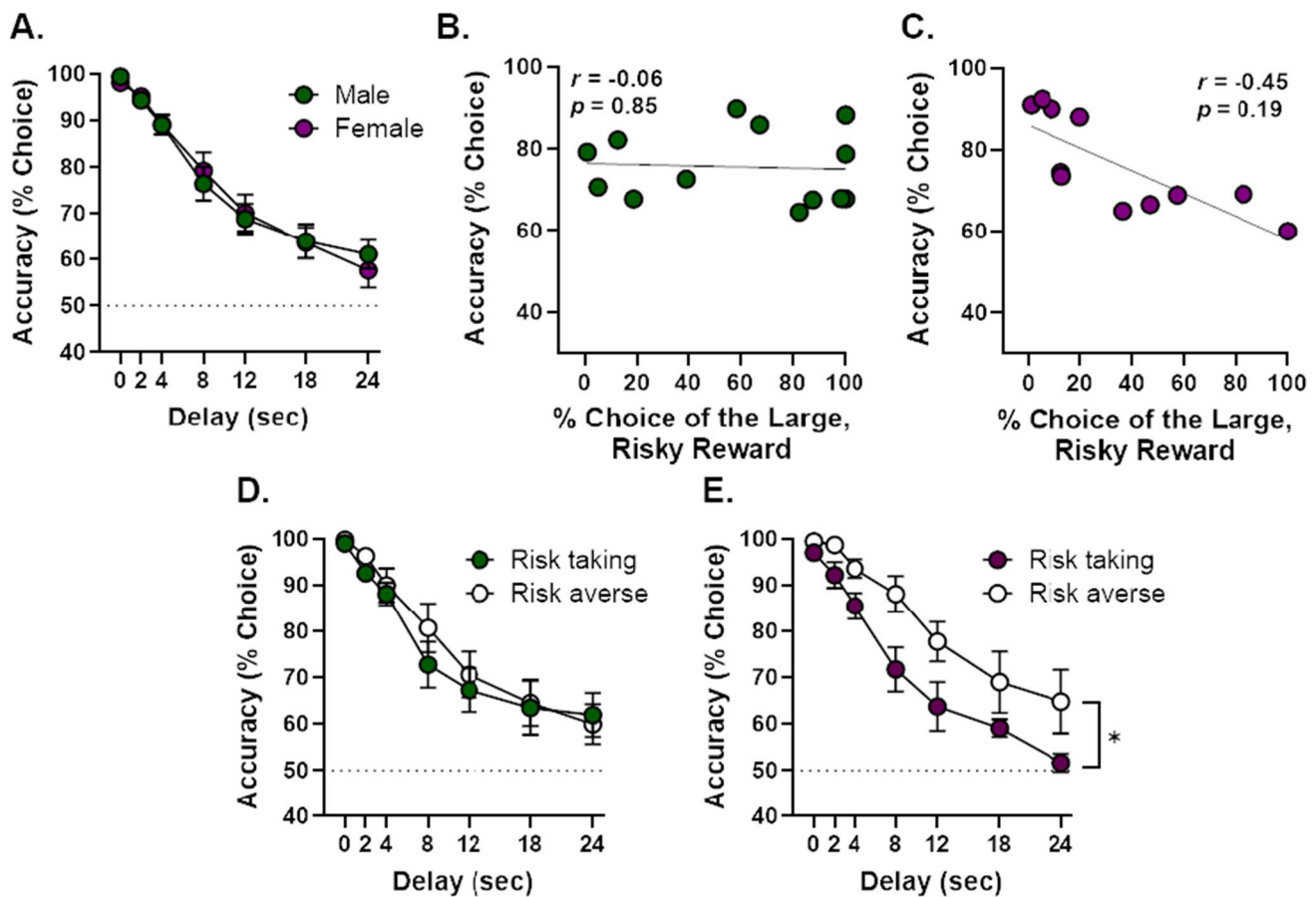
Fig. 4 *Performance on the Probability Discounting Task in Male and Females.* **A.** There were no differences between males and females in choice of the large, uncertain reward. **B, C.** There was no significant correlation between choice of the large, risky reward in the Risky Decision-making Task and choice of the large, uncertain reward in the Probability Discounting Task in males (**B**) or females (**C**). **D.** Risk-averse males chose the large, uncer- tain reward significantly more than risk-taking males. **E.** There were no differences between risk-taking and risk-averse females in choice of the large, uncertain reward. For A, D, and E, data are represented as mean ($\pm$) standard error of the mean. Asterisks indicate $p < 0.05$. For B and C, each data point represents the aver- age choice of the large reward across trial blocks 2–5 on each task for each individual rat

F (6, 132) = 124. 33, $p < 0.01$, $\eta^2 = 0.85$; day X delay, F (24, 528) = 0.57, $p = 0.95$, $\eta^2 = 0.03$] in both sexes [day X sex, F (4, 88) = 0.44, $p = 0.78$, $\eta^2 = 0.02$; day X delay X sex, F (24, 528) = 1.27, $p = 0.18$, $\eta^2 = 0.05$]. A two- factor repeated-measures ANOVA revealed that choice accuracy was comparable between males and females [Fig. 5; sex, F (1, 22) < 0.01, $p = 1.00$, $\eta^2 < 0.01$], with less accurate choice at longer delays in both sexes [delay, F (6, 132) = 124.33, $p < 0.01$, $\eta^2 = 0.85$; sex X delay, F (6, 132) = 0.51, $p = 0.80$, $\eta^2 = 0.02$]. Females did, how- ever, complete significantly fewer trials than males [t (22) = 3.34, $p < 0.01$, d = 1.37] and differed in their laten- cies to press each lever. Specifically, in addition to hav- ing longer latencies overall [F (1, 22) = 19.27, $p < 0.01$, $\eta^2 = 0.47$], females took significantly longer than males to press the incorrect lever [sex X lever identity, F (1, 22) = 5.45, $p = 0.03$, $\eta^2 = 0.20$].

Relationship between performance on the RDT and the Delayed Response Working Memory Task

Correlational analyses revealed that risk taking in the RDT was not significantly associated with choice accuracy on the Delayed Response Working Memory Task in males (Fig. 5B; $r = -0.07$, $p = 0.83$) or in females (Fig. 5C; $r = 0.16$, $p = 0.65$). Similarly, risk taking was not significantly correlated with the number of trials completed in the working memory task in either sex (males: $r = 0.27$, $p = 0.40$; females: $r = 0.35$, $p = 0.33$). A comparison of choice accuracy between risk- taking and risk-averse rats revealed that risk-averse females were more accurate relative to risk-taking females [Fig. 5E; delay, F (6, 54) = 63.76, $p < 0.01$, $\eta^2 = 0.88$; group, F (1, 9) = 6.76, $p = 0.03$, $\eta^2 = 0.51$; group X delay, F (6, 54) = 1.50, $p = 0.20$, $\eta^2 = 0.14$]. In contrast, there were no differences in choice accuracy between risk-taking and risk-averse males

Fig. 5 *Performance on the Delayed Working Memory Task in Males and Females.* **A**. There were no differences between males and females on accuracy (percent choice correct). **B**. There was no significant correlation between choice of the large, risky reward in the Risky Decision-making Task and accuracy in the Delayed Working Memory Task in males (**B**) or females (**C**). **D**. There were no differences between risk-taking and risk-averse males in accuracy in the Delayed Working Memory Task. **E**. Risk-averse females displayed significantly greater accuracy in the Delayed Working Memory Task than risk-taking females. For A, D, and E, data are represented as mean ($\pm$) standard error of the mean. Asterisks indicate $p < 0.05$. For B and C, each data point represents the average choice of the large reward across trial blocks 2–5 in the RDT and the average accuracy across all delays in the Delayed Working Memory Task for each individual rat

[Fig. 5D; delay, $F(6, 66) = 61.24$, $p < 0.01$, $\eta^2 = 0.85$; group, $F(1, 11) = 0.30$, $p = 0.60$, $\eta^2 = 0.03$; group X delay, $F(6, 66) = 0.62$, $p = 0.71$, $\eta^2 = 0.05$]. The number of completed trials did not differ between risk groups in males [$t(11) = 0.82$, $p = 0.43$, d = 0.46] or females [$t(9) = 0.13$, $p = 0.89$, d = 0.08].
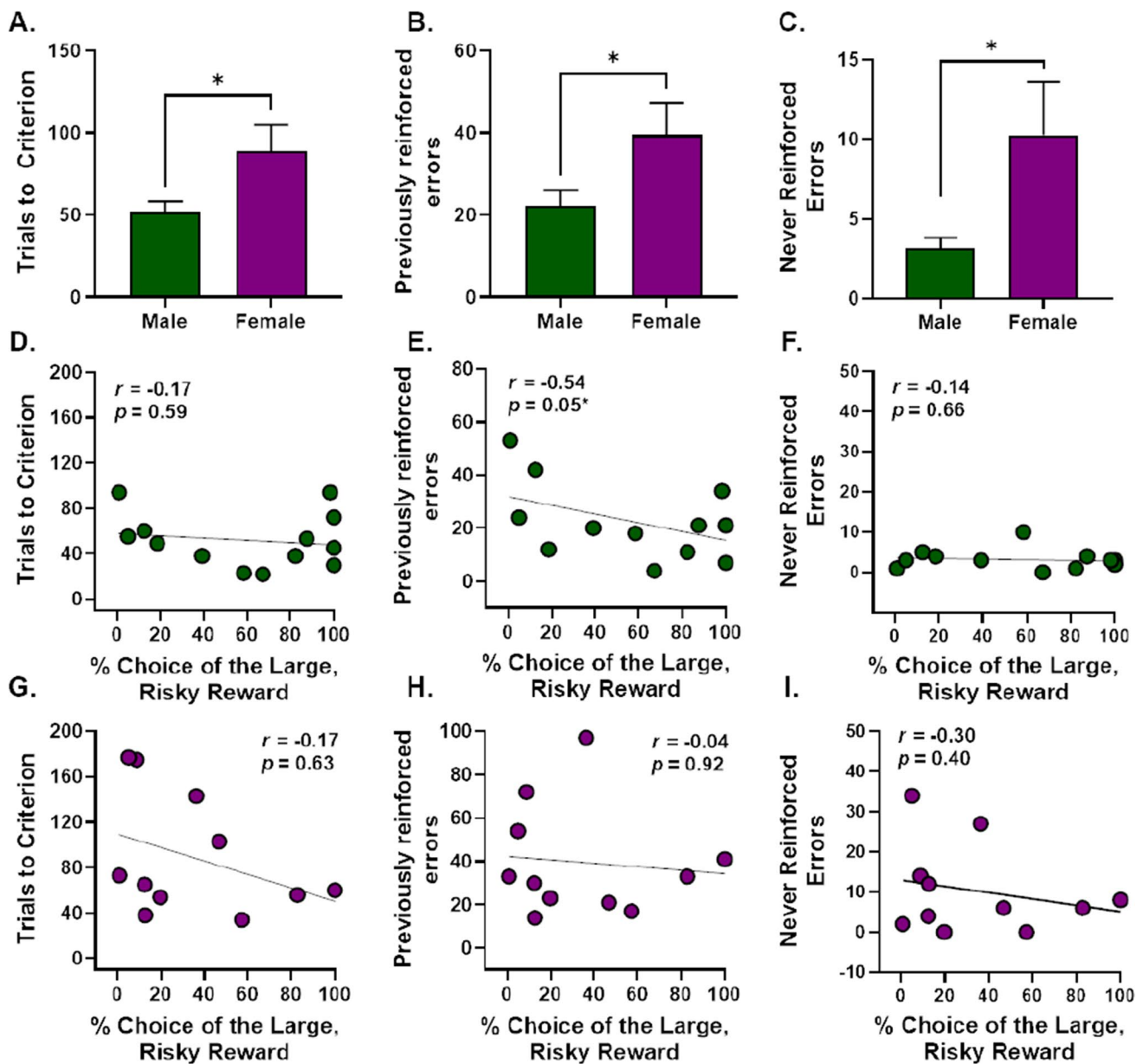
Set-shifting Task

Performance on the Set-shifting Task

On the initial discrimination, males and females did not differ in the number of trials to reach criteria [male: 346.27 ± 55.13 (mean $\pm$ standard error of the mean); female: 272.55 ± 43.08; $t(22) = 1.03$, $p = 0.32$, d = 0.42], the number of errors committed [male: 140.54 ± 23.01; female: 118.55 ± 27.92; $t(22) = 0.61$, $p = 0.55$, d = 0.56]. On the set shift, however, females required significantly more trials to reach criterion

than males [Fig. 6A; $t(22) = -2.29$, $p = 0.03$, d = 0.94] and made significantly more previously reinforced [Fig. 6B; $t(22) = -2.12$, $p = 0.05$, d = 0.87] and never reinforced errors [Fig. 6C; $t(22) = -2.27$, $p = 0.03$, d = 0.93] than males.

Relationship between performance on the RDT and the Set-shifting Task

Risk taking in the RDT was not significantly correlated with the number of trials to reach criteria in the initial discrimination in males ($r = -0.03$, $p = 0.92$) or females ($r = -0.45$; $p = 0.19$), nor was it correlated with the number of errors committed in these sessions (males, $r = -0.04$, $p = 0.90$; females, $r = -0.42$, $p = 0.23$). On the set shift, risk taking in males was not significantly correlated with the number of trials to reach criterion (Fig. 6D; $r = -0.17$, $p = 0.59$) or the number of never reinforced

Fig. 6 *Performance on the Set-shifting Task in Male and Females.* **A-C.** Females required significantly more trials to reach criterion and made significantly more previously reinforced and never reinforced errors on the set shift than males. **D.** There was no significant correlation between choice of the large, risky reward in the Risky Decision-making Task (RDT) and trials to reach criterion in males. **E.** Greater choice of the large, risky reward in the RDT was significantly correlated with fewer previously reinforced errors in males. **F.** There was no significant correlation between choice of the large, risky reward in the RDT and the number of never reinforced errors in males. **G-I.** There were no significant correlations between choice of the large, risky reward in the RDT and any of the behavioral measures on the set shift in females. For A-B, data are represented as mean ($\pm$) standard error of the mean. Asterisks indicate $p < 0.05$. For D-I, each data point represents the average choice of the large reward across trial blocks 2–5 in the RDT and the mean number of trials to criterion or number of errors on the set shift for each individual rat

errors (Fig. 6F; $r = -0.14$, $p = 0.66$), but it was significantly correlated with the number of previously reinforced errors (Fig. 6E; $r = -0.54$, $p = 0.05$), with greater risk taking associated with fewer previously reinforced errors. In females, risk taking was not significantly correlated with the number of trials to reach criterion (Fig. 6G;

$r = -0.17$, $p = 0.63$), the number of never reinforced errors (Fig. 6I; $r = -0.30$, $p = 0.40$) or the number of previously reinforced errors (Fig. 6H; $r = -0.04$, $p = 0.92$) on the set shift. There were no differences in the number of trials to reach criteria during the initial discrimination between risk-taking and risk-averse rats in either sex [males: t
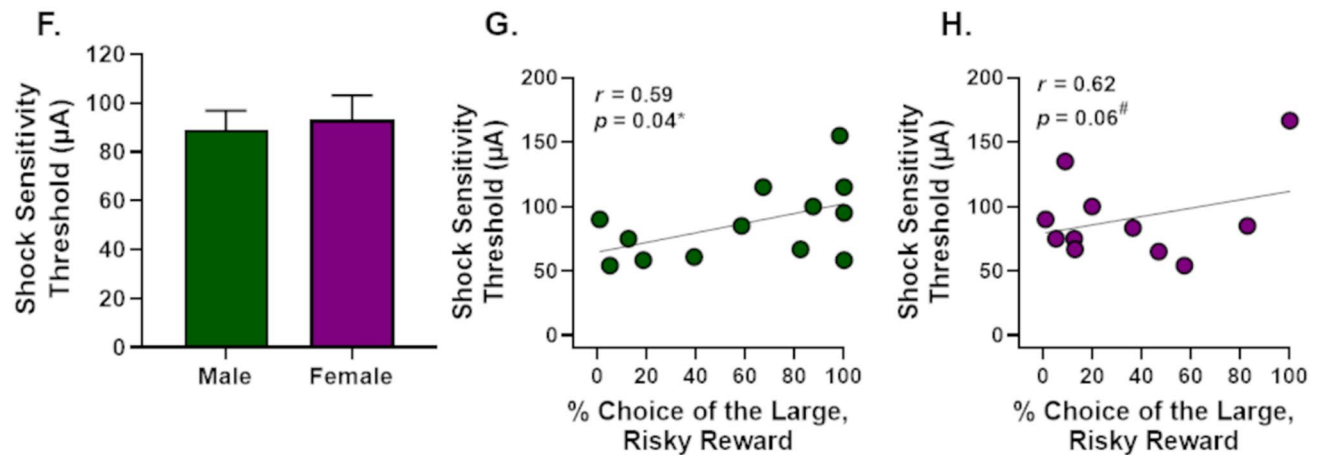
Progressive Ratio Schedule of Reinforcement
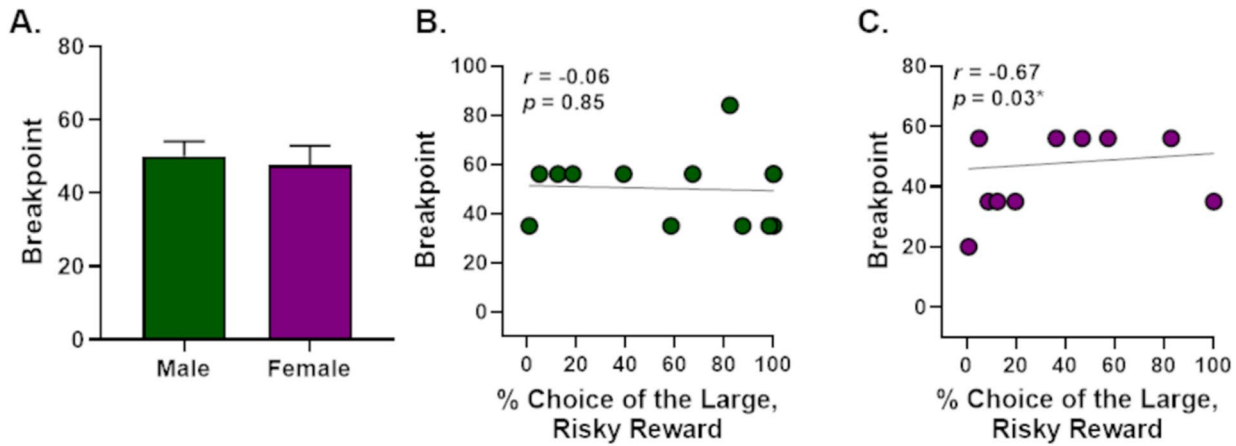
Differential Reinforcement of Low Rates of Responding
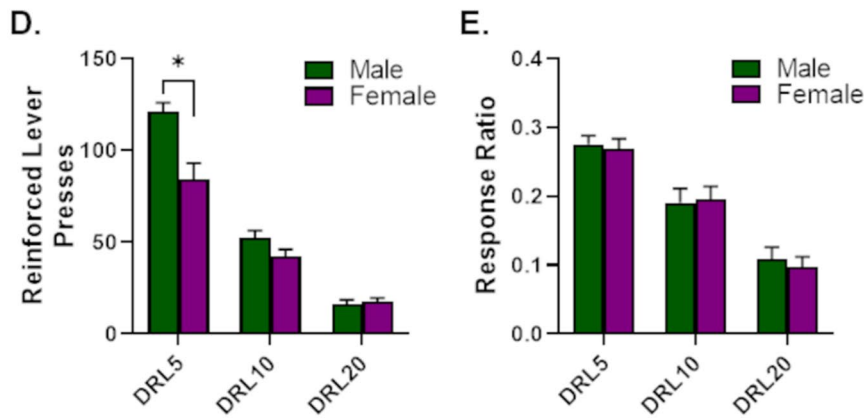
Shock Reactivity Thresholds

◄**Fig. 7** *Performance on the Progressive Ratio Schedule of Reinforcement Task, Differential Reinforcement of Low Rates of Responding Task and Shock Reactivity Assays in Male and Females.* **A.** There was no difference between males and females in breakpoint (the ratio at which rats ceased to lever press for food rewards). **B.** There was no significant correlation between choice of the large, risky reward in the Risky Decision-making Task (RDT) and breakpoint in males. **C.** Higher breakpoints were associated with fewer choices of the large, risky reward (i.e., greater risk aversion) in the RDT in females. **D.** There were no significant differences between males and females in response ratios on any of the reinforcement schedules. **E.** Males made significantly more reinforced lever presses than females on the Differential Reinforcement schedule 5, but there were no sex differences in reinforced lever presses on any other reinforcement schedule. **F.** There were no sex differences in shock reactivity thresholds. **G.** Greater choice of the large, risky reward in the RDT was significantly correlated with higher shock reactivity thresholds in males. **H.** There was a near significant correlation between choice of the large, risky reward in the RDT and shock reactivity thresholds in females, with greater choice of the large, risky reward associated with higher shock reactivity thresholds. For A, D-F, data are represented as mean ($\pm$) standard error of the mean. For B, C, G and H, each data point represents the average choice of the large reward across trial blocks 2–5 in the RDT and the mean value of the dependent variable of interest for each individual rat. Asterisks indicate $p < 0.05$

$(11) = -0.68$, $p = 0.51$, d = 0.38; females: $t(9) = 0.90$, $p = 0.39$, d = 0.50] nor were there differences between these groups in the number of trials to reach criterion [males: $t(1, 11) = -0.19$, $p = 0.85$, d = 0.11; females: $t(9) = -0.95$, $p = 0.37$, d = 0.58], the number of previously reinforced errors [males: $t(11) = -1.53$, $p = 0.16$, d = 0.85; females: $t(9) = -0.12$, $p = 0.91$, d = 0.07] or never reinforced errors [males: $t(11) = -1.71$, $p = 0.12$, d = 0.95; females: $t(9) = -0.79$, $p = 0.45$, d = 0.48] on the set shift.

Progressive Ratio Schedule of Reinforcement Task

Performance on the Progressive Ratio Schedule of Reinforcement Task

Vendor was a significant covariate in the analyses of all measures on this assay [$ps < 0.05$, $\eta^2 s < 0.30$], with Charles River rats exhibiting greater motivation to work for food than Envigo rats. Hence, analyses of sex differences in measures of food motivation included vendor as a covariate. These analyses revealed that males and females did not differ in the number of lever presses [$F(1, 21) = 0.15$, $p = 0.70$, $\eta^2 < 0.01$], amount of food earned [$F(1, 21) = 0.39$, $p = 0.54$, $\eta^2 = 0.02$] or their breakpoint [Fig. 7A; $F(1, 21) = 0.22$, $p = 0.64$, $\eta^2 = 0.01$] on this assay.

Relationship between performance on the RDT and the Progressive Ratio Schedule of Reinforcement Task

In males, there was no correlation between risk taking in the RDT and the number of lever presses ($r = 0.03$, $p = 0.92$), amount of food earned ($r = -0.10$, $p = 0.76$) or breakpoint

(Fig. 7B; $r = -0.06$, $p = 0.85$). At first glance, Fig. 7C would seem to indicate that there were no significant correlations between risk taking and PR measures in females; however, correlational analyses (when controlling for vendor) revealed a significant association between risk taking and breakpoint ($r = -0.67$, $p = 0.03$) as well as between risk taking and number of lever presses ($r = -0.70$, $p = 0.03$). The correlation between risk taking and amount of food earned did not quite reach statistical significance in females ($r = -0.58$, $p = 0.08$). When comparing behavioral measures on the PR assay between risk-taking and risk-averse rats, vendor was a significant covariate for both males and females [$ps < 0.05$, $\eta^2 s < 0.30$] and was thus included as a covariate in these analyses. There were no differences in the number of lever presses [$F(1, 10) = 0.24$, $p = 0.64$, $\eta^2 = 0.02$], amount of food earned [$F(1, 10) < 0.01$, $p = 1.00$, $\eta^2 < 0.01$] or breakpoint [$F(1, 10) = 0.02$, $p = 0.90$, $\eta^2 < 0.01$] between risk-taking and risk-averse male rats. Similarly, despite the significant correlations between risk taking and measures of food motivation in females, there were no differences in number of lever presses [$F(1, 8) = 2.76$, $p = 0.14$, $\eta^2 = 0.25$], amount of food earned [$F(1, 8) = 2.64$, $p = 0.14$, $\eta^2 = 0.15$] or breakpoint [$F(1, 8) = 1.45$, $p = 0.26$, $\eta^2 = 0.26$] between risk-taking and risk-averse female rats.

Differential Reinforcement of Low Rates of Responding Task

Performance on the Differential Reinforcement of Low Rates of Responding Task

Although male rats made significantly more reinforced lever presses than females on DRL5 [Fig. 7D; $t(22) = 3.88$ $p < 0.01$, d = 1.59], there were no sex differences in the number of sessions to reach criteria [male: 7.30 ± 0.54; female: 7.64 ± 0.70; $t(22) = -0.38$, $p = 0.71$, d = 0.16] or the ratio of correct responses [Fig. 7E; $t(22) = 0.26$, $p = 0.80$, d = 0.11] for this delay schedule. There were also no sex differences in the number of sessions to reach criteria [male: 11.77 ± 1.50; female: 11.64 ± 1.96; $t(22) = 0.06$, $p = 0.96$, d = 0.02], reinforced lever presses [$t(22) = 1.09$, $p = 0.29$, d = 0.45] or ratio of correct responses [$t(22) = -0.24$, $p = 0.82$, d = 0.10] on DRL10. Finally, there were no sex differences in the number of sessions to reach criteria [male: 13.75 ± 1.91; female: 13.00 ± 2.80; $t(19) = 0.23$, $p = 0.82$, d = 0.10], reinforced lever presses [$t(19) = -0.50$, $p = 0.62$, d = 0.22] or ratio of correct responses [$t(19) = 0.48$, $p = 0.64$, d = 0.21] on DRL20.

Relationship between performance on the RDT and the Differential Reinforcement of Low Rates of Responding Task

To examine the relationship between performance on the RDT and performance on the DRL, risk taking was

Table 4 Comparison of performance in the Differential Reinforcement of Low Rates of Responding Task between risk-taking and risk-averse rats

	Factor(s)	F- or t-value	p-value	Effect size (η^2 or d)
DRL5				
Males:				
Risk-taking vs. risk-averse	Reinforced lever presses	$F (1,10) = 0.20$	0.66*	0.02
	Sessions to criteria	$t (11) = -0.04$	0.97	0.02
	Ratio of correct responses	$t (11) = -1.15$	0.27	0.64
Females:				
Risk-taking vs. risk-averse	Reinforced lever presses	$t (9) = -0.71$	0.50	0.43
	Sessions to criteria	$t (9) = -0.20$	0.85	0.12
	Ratio of correct responses	$t (9) = -1.08$	0.31	0.65
DRL10				
Males:				
Risk-taking vs. risk-averse	Reinforced lever presses	$t (11) = -0.70$	0.50	0.39
	Sessions to criteria	$t (11) = -0.14$	0.89	0.08
	Ratio of correct responses	$t (11) = -1.10$	0.29	0.61
Females:				
Risk-taking vs. risk-averse	Reinforced lever presses	$t (9) = -0.93$	0.38	0.57
	Sessions to criteria	$t (9) = -1.22$	0.25	0.74
	Ratio of correct responses	$t (9) = -1.40$	0.20	0.85
DRL20				
Males:				
Risk-taking vs. risk-averse	Reinforced lever presses	$t (10) = -0.35$	0.73	0.20
	Sessions to criteria	$t (10) = -0.29$	0.78	0.17
	Ratio of correct responses	$t (10) = -0.12$	0.91	0.07
Females:				
Risk-taking vs. risk-averse	Reinforced lever presses	$t (7) = -0.57$	0.59	0.38
	Sessions to criteria	$t (7) = -1.81$	0.11	1.21
	Ratio of correct responses	$t (7) = -0.45$	0.67	0.30

*Vendor included as a covariate [$F (1, 10) = 6.06$, $p = 0.03$, $\eta^2 = 0.38$]

correlated with the three main dependent variables of this task on the three different delays separately for each sex. Consequently, p-values were Bonferroni-adjusted to account for the significant number of correlations. In males, there were no significant correlations between risk taking in the RDT and performance on the DRL task on DRL5 (reinforced lever presses: $r = -0.12$, $p = 0.72$; sessions to criteria, $r = 0.15$, $p = 0.67$; ratio of correct responses, $r = -0.24$, $p = 0.49$), DRL10 (reinforced lever presses: $r = -0.09$, $p = 0.80$; sessions to criteria, $r = 0.20$, $p = 0.56$; ratio of correct responses, $r = -0.27$, $p = 0.42$) or DRL20 (reinforced lever presses: $r = -0.18$, $p = 0.60$; sessions to criteria, $r = 0.09$, $p = 0.80$; ratio of correct responses $r = 0.07$, $p = 0.84$). There were also no significant correlations between risk taking in females and their performance on the DRL task on DRL5 (reinforced lever presses: $r = 0.30$, $p = 0.47$; sessions to criteria, $r = 0.18$, $p = 0.66$; ratio of correct responses, $r = 0.09$, $p = 0.83$), DRL10 (reinforced lever presses: $r = -0.08$, $p = 0.86$;

sessions to criteria, $r = 0.24$, $p = 0.56$; ratio of correct responses, $r = -0.44$, $p = 0.27$) or DRL20 (reinforced lever presses: $r = -0.37$, $p = 0.36$; sessions to criteria, $r = -0.17$, $p = 0.69$; ratio of correct responses, $r = -0.38$, $p = 0.35$). Because there were no significant correlations between risk taking and performance on the DRL at any schedule, these results are not presented graphically. Consistent with the lack of correlations between performance on these tasks, there were no differences between risk-taking and risk-averse rats (male or female) on behavioral measures of the DRL task (Table 4).

Shock reactivity thresholds

There were no sex differences in shock reactivity thresholds [Fig. 7F; $t (22) = -0.29$, $p = 0.77$]. Correlational analyses revealed that greater risk taking in males was associated with higher shock reactivity thresholds (Fig. 7G; $r = 0.59$, $p = 0.04$). A similar relationship existed for

females, although it did not quite reach statistical significance (Fig. 7H; $r = 0.62$, $p = 0.06$). Shock reactivity thresholds were compared between risk-taking and risk-averse rats, with vendor included as a covariate for males [F (1, 10) = 8.89, $p = 0.01$, $\eta^2 = 0.47$]. These analyses showed that risk-taking male rats had higher shock reactivity thresholds than risk-averse male rats [F (1, 10) = 8.61, $p = 0.02$, $\eta^2 = 0.46$], consistent with the results of the correlational analyses. There were, however, no differences between risk-taking and risk-averse female rats in their shock reactivity thresholds [t (9) = 0.19, $p = 0.85$, d = 1.16]. Additional analyses were conducted to examine whether locomotor activity during shock delivery during the RDT correlated with shock reactivity thresholds. In males, there was a near-significant correlation between these variables ($r = 0.53$, $p = 0.06$), with greater locomotor activity during the shock delivery associated with higher shock reactivity thresholds. In contrast, there was no correlation between these variables in females ($r = 0.03$, $p = 0.94$).

Discussion

Extremes in risk taking (either excessively low or high levels) are associated with neuropsychiatric diseases including substance use disorder (excessive risk taking; Chen et al., 2020) and eating disorders (excessive risk aversion; Kaye et al., 2013). To better understand the cognitive and behavioral mechanisms that underlie risk taking, previous studies have examined the relationship between individual differences in risk taking and other cognitive and affective mechanisms (Gabriel et al., 2019; Shimp et al., 2015). Although these studies have been informative, they are constrained by the fact that only male subjects were used, despite well-established sex differences in risk taking (Liley et al., 2019; Orsini et al., 2022; Orsini et al., 2016; van den Bos et al., 2013). Hence, the objective of the current study was to compare the cognitive mechanisms that might mediate risk taking between male and female rats. Male and female rats were first characterized on the Risky Decision-making Task (RDT) and then underwent a series of cognitive and behavioral assays. Not only were sex differences quantified on each assay, but relationships between risk taking in the RDT and performance on each assay were examined. Consistent with previous work (Blaes et al., 2022; Orsini et al., 2016), males preferred the large, risky reward to a greater extent than females. When risk taking in males and females was evaluated with respect to performance on other assays, sex-specific behavioral profiles emerged. These findings complement those of prior studies and provide a more comprehensive understanding of the cognitive and behavioral mechanisms that contribute to sex differences in risk taking.

Sex differences in risk taking

The current study replicated the sex differences in the RDT first reported by Orsini et al. (2016). The data presented here, however, provide additional insight into the interpretation of these differences. Analysis of win-stay and lose-shift behavior, which were not quantified in Orsini et al. (2016), revealed that females displayed greater lose-shift behavior, indicative of enhanced sensitivity to the punished outcome. This observation is consistent with other work showing that females acquire avoidance learning at a faster rate than males (Chowdhury et al., 2019) and bias choice away from delayed punished rewards (Liley et al., 2019). Although Orsini et al. (2016) provided evidence that greater risk aversion in females cannot be attributed to differences in shock perception, they did not assess reactivity to punishment outside of the RDT. To more definitively address this issue, males and females were assessed in a well-established assay used to identify a rat's threshold to detect footshock. Importantly, there were no differences in shock reactivity thresholds between males and females. Risk taking in the RDT was positively correlated with shock reactivity thresholds in both sexes; surprisingly, the relationship appeared to be slightly stronger in males than females as the comparison of shock reactivity thresholds between risk-taking and risk-averse rats was only significant in males. Collectively, these data provide a strong argument against the interpretation that greater risk aversion and sensitivity to the punished outcome in females are simply due to augmented perception of a footshock relative to males.

Another alternative explanation for greater risk aversion in females is that they are less motivated to work for food. Orsini et al. (2016) addressed this in several ways, such as testing rats on fixed ratio (FR) schedules of reinforcement. Although males made more lever presses than females at higher FR schedules, performance on the RDT did not correlate with lever pressing at any FR schedule in males or females, leading to the conclusion that sex differences in risk taking could not solely be due to differences in motivation to work for food. Unlike FR schedules, progressive ratio (PR) schedules of reinforcement require the subject to complete an increasing number of lever presses for the next food reward within a test session. The use of a PR schedule may therefore be a more accurate method to determine whether sex differences in risk taking are related to sex differences in their willingness to incur an increasing cost (effort) to obtain a food reward. Consequently, in the current study, males and females were tested on a PR assay wherein the number of lever presses required to obtain a single food reward increased within the test session. In contrast to the findings of Orsini et al. (2016), there were no differences between males and females in

performance on the PR task. Although there was no correlation between risk taking and any PR behavioral measure, there was a significant relationship between these variables in females, with greater risk aversion associated with a greater motivation to work for food. The direction of this relationship offers additional support for the assertion that reduced risk taking in females is independent of motivation for food. In fact, it could suggest that biasing choice away from risky options may have greater motivational value to females compared with males.

Sex differences in other cognitive measures

Unlike risk taking, there were no sex differences in other forms of choice behavior. On the Delay Discounting Task, which assesses impulsive choice, males and females discounted the large, delayed reward to a similar extent. Although this is incongruous with recent work showing that females are more impulsive than males (Hernandez et al., 2020b), it is consistent with other studies reporting a lack of sex differences in impulsive choice (Eubig et al., 2014; Lukkes et al., 2016; Perry et al., 2008; Sackett et al., 2019). Complicating the matter further, Panfil et al. (2020) have reported that males are more impulsive than females. Possible explanations for these discrepancies between studies include the use of different strains of rats [e.g., Long-Evans rats in the current study; Fischer 344 X Brown Norway F1 hybrid rats in Hernandez et al. (2020b); Sprague–Dawley rats in Panfil et al. (2020)] and differences in the structure of and parameters used in the behavioral assay (e.g., delay duration, delays associated with one lever or both levers, etc.). Alternatively, choice of the large, delayed reward may not be a sensitive enough behavioral measure to detect sex differences. Despite the inconsistency between the current study and that of Hernandez et al. (2020b) in the effects of sex on this measure, both studies found that females exhibited longer latencies to press levers during forced choice trials and made significantly more omissions than males. Hence, future studies evaluating sex differences in impulsive choice should consider including analyses of these ancillary behavioral measures as they may be more sensitive to differences between males and females in this form of decision making.

Similar to performance on the Delay Discounting Task, there were no sex differences in performance on the Probability Discounting Task. These findings are in contrast to a recent study showing that males prefer the large, uncertain reward more than females on the Probability Discounting Task (Islas-Preciado et al., 2020). The reason for this discrepancy in findings is not entirely clear, but it could be attributable to the fact that rats had significantly more experience in the operant chambers in the current study before being tested on the Probability Discounting Task relative to

the rats in the study by Islas-Preciado et al. (2020). Future studies are therefore required to confirm either the presence (Islas-Preciado et al., 2020) or absence (current study) of sex differences in this form of risky decision making.

In addition to decision making, working memory capacity and cognitive flexibility were also compared between males and females. Contrary to recent findings from Blaes et al. (2019) in which females were less accurate on the Delayed Response Working Memory Task, there was no difference in choice accuracy between males and females in the current study. An absence of sex differences, however, is consistent with other studies reporting a lack of sex differences in performance on other tasks that tax working memory capacity (Healy et al., 1999; Hernandez et al., 2020a), although these tasks were structured differently and relied more heavily on spatial navigation and memory relative to the Delayed Response Working Memory Task. In contrast to working memory performance, there were pronounced sex differences in performance on the Set-shifting Task, which was used to assess cognitive flexibility. Specifically, females required significantly more sessions to reach criterion on the set shift and made significantly more errors compared with males, suggesting that females are less cognitively flexible than males. A recent meta-analysis of studies that evaluated sex differences in executive function in humans reported that men and women do not differ in performance on a set-shifting task (Gaillard et al., 2021). Similarly, although female mice exhibit longer latencies to complete trials, they otherwise perform comparably to males on a behavioral task used to assess cognitive flexibility (Bissonette et al., 2012). Using a set-shifting task similar to that used in the current study, Chowdhury et al. (2019) also reported a lack of sex differences in cognitive flexibility in rats. Finally, on a set-shifting task modified for use in non-human primates, LaClair et al. (2019) did not observe sex differences in measures of cognitive flexibility. Results from a more recent study in mice, however, are consistent with the sex differences observed in the current study: on the set shift, female mice made significantly more previously reinforced errors than males (Anderson et al., 2021). Considered together, the findings supporting sex differences in cognitive flexibility remain equivocal. Besides the studies reviewed here, there are in fact very few studies of cognitive flexibility that include both males and females, and of those that have included both sexes (including those mentioned above), the tasks used to assess cognitive flexibility and parameters therein differ considerably [e.g., use of operant chamber with levers (current study) vs. use of open test arena with bowls]. There are also other forms of cognitive flexibility, such as reversal learning, in which subjects are required to adjust their actions based on changes in reward contingencies within a session (as opposed to between sessions as in the Set-shifting Task). Not surprisingly, there are also sex differences in reversal

learning wherein females are less sensitive to the feedback of a preceding trial to alter choice in the subsequent trial (Bryce & Floresco, 2021). These observations are consistent with the findings of the current study in which females made more errors than males, indicative of their inability to use new information about contingency rules to alter ongoing behavior. Future studies are necessary to determine whether the relationship between risk taking and cognitive flexibility (as measured on a Set-shifting Task) extends to other forms of cognitive flexibility.

Sex differences in relationships between risk taking and other cognitive measures

One of the primary objectives of the current study was to examine the relationship between risk taking and other cognitive measures in both males *and* females and whether such relationships account for sex differences in risk taking. Although there were no significant correlations between risk taking and working memory in either sex, a comparison between risk-taking and risk-averse rats on working memory performance revealed that risk-averse females had greater choice accuracy compared with risk-taking females. In contrast, there were no differences in choice accuracy between risk-taking and risk-averse males, consistent with previous work (Shimp et al., 2015). These findings suggest that risk aversion in females may be related to greater working memory capacity. There are several potential interpretations of the unique relationship between risk aversion and working memory performance in females. First, better recall of proximal and recent potential threats may be more evolutionarily adaptive for female rats, who are the primary caregivers for offspring. Avoidance of risk could enhance females' reproductive success by evading potential harm and death not only for themselves but also for their offspring. A second, but not mutually exclusive, interpretation is that risk-averse females are more anxious than risk-taking females and it is this increased anxiety that improves working memory in this group of females. Indeed, recent work has shown that high levels of anxiety improve working memory (Charpentier et al., 2016) and that individuals with anxiety disorder exhibit greater risk avoidant behavior (Charpentier et al., 2017; Maner & Schmidt, 2006). Further, others have shown that better working memory in aged male rats is correlated with augmented hypothalamic-pituitary axis activity (McQuail et al., 2018), a physiological phenomenon also associated with increased anxiety (Tafet & Nemeroff, 2020). Although this observation has only been reported in males, it is still notable because, relative to young adult males, aged male rats exhibit greater risk aversion (Dragone et al., 2019), similar to phenotypical female risk-taking behavior, providing support (albeit indirect) for a role for anxiety in mediating the relationship between risk aversion and better working

memory. Despite the fact that performance on the RDT and measures of anxiety (e.g., performance on the Elevated Plus Maze) are not correlated in males (Simon et al., 2011), this relationship has not been directly examined in females, but such information would be helpful to fully understand the nature of the association between risk aversion and working memory performance in females. Relationships with anxiety notwithstanding, these findings suggest that female risk taking is related to the ability to retain information about recent aversive outcomes in working memory to guide future choice behavior.

In addition to working memory capacity, cognitive flexibility was also evaluated as a function of performance on the RDT. There was a significant correlation between risk taking and cognitive flexibility wherein greater risk taking was associated with fewer previously reinforced errors. Similar to the relationship between working memory and risk taking, this association was sex-dependent, but in contrast to the relationship between working memory and risk taking, it was specific to males and not females. These results suggest that greater risk taking in males is associated with better cognitive flexibility, reproducing findings from others who also used the RDT and the same Set-shifting Task to evaluate similar cognitive relationships in males (Shimp et al., 2015). When considering the fact that individuals with substance use disorders or pathological gambling exhibit increased risk taking and impaired cognitive flexibility (Verdejo-Garcia et al., 2015), the direction of this relationship seems counterintuitive. In support of this relationship, however, several studies have shown that enhanced reward sensitivity during tasks involving gains and losses is associated with better cognitive control (van Duijvenvoorde et al., 2016). Further, Lawrence et al. (2008) reported that entrepreneurs who were more likely to take risks displayed superior cognitive flexibility relative to those who were less likely to take risks. Given the finding that greater risk taking is associated with better cognitive flexibility in drug-naïve rats, it suggests that chronic exposure to drugs or continual problematic gambling behavior may alter the nature of this relationship such that greater cognitive *inflexibility* becomes subsequently associated with greater risk taking.

Despite associations between risk taking and measures of working memory capacity and cognitive flexibility, there were no relationships between risk taking and impulsive choice or impulsive action. The lack of a relationship between risk taking and impulsive choice is consistent with prior work conducted in male rats (Gabriel et al., 2019; Shimp et al., 2015) and now extends to female choice behavior. A previous study, however, reported that increased risk taking is associated with elevated impulsive action, which directly contrasts with findings of the current study. It is difficult to determine the source of the discrepancy between the findings of the two studies given that many of the factors that could typically account for

these differences, such as strain or task structure, were comparable across studies. Given the vendor differences that were observed in other behavioral measures, it is possible that differences between studies are due to procurement of rats from different vendors. However, like the current study, Gabriel et al. (2019) also obtained their subjects from both Envigo and Charles River. Sample sizes (when controlling for vendor) for males and females were smaller in the current study relative to those in the study by Gabriel et al. (2019); it is therefore conceivable that with larger sample sizes for each sex, significant relationships would emerge. Another potential (and not mutually exclusive) explanation could be the order in which the behavioral tasks was conducted. Whereas testing on the DRL occurred after testing on the PR assay in the current study, testing on the DRL relative to other behavioral tasks varied across several cohorts in the study by Gabriel et al. (2019). Hence, additional experiments in which the sequence of behavioral testing is counterbalanced across groups of rats are necessary to resolve the discrepancy between these studies.

Finally, there was no correlation between risk taking in the RDT and risky choice in the Probability Discounting Task in males or females. A comparison of performance on the Probability Discounting Task between risk-taking and risk-averse rats (based on their risk preference in the RDT), however, revealed that risk-taking male rats chose the large, uncertain reward in the Probability Discounting Task significantly less than risk-averse male rats. These results were unexpected as a previous study using only male rats reported that greater risk taking on the RDT predicted greater risky choice in the Probability Discounting Task (rats were not divided into subgroups based on risk preference in this study; Simon et al., 2009). Because Long-Evans rats were used in both studies, it is unlikely that the conflicting results are due to strain differences. One potential explanation is that the experimental history of the rats differed at the time of testing on the Probability Discounting Task between the two studies. In the current study, rats were tested on the RDT and the Delay Discounting Task prior to being tested on the Probability Discounting Task whereas in the study by Simon et al. (2009), rats received injections of amphetamine during testing on the RDT, which decreased rats' choice of the large, risky reward, before being re-trained on the RDT and progressing to the other two decision-making tasks. Alternatively, differences between studies could be due to the fact that rats in each study originated from different geographic locations, despite the similarity of vendor for some rats (Charles River). Indeed, there are significant differences in the ability to induce seizures and in neurotransmitter levels in the hippocampus between rats of the same strain and vendor but from different geographic locations (Brandt et al., 2016; Portelli et al., 2009). Such variability could be due to small differences at each location, such as the composition of the chow provided to the rats, or larger procedural differences, such as the light/

dark cycle in the colony rooms. These factors can have a long-lasting impact on the physiology of a rodent and, as a consequence, may lead to divergent behavioral phenotypes.

As alluded to previously, one limitation of this study is that all subjects were tested in the various behavioral assays in the same order and in the same operant chambers across the entire study. It is therefore possible that there was carryover of learning between tasks, resulting in performance that may differ from performance when the tasks are presented in a different or random order. To mitigate the impact of prior learning on performance in subsequent tasks, rats underwent extensive remedial training before proceeding to the subsequent task wherein they were re-trained to engage with both levers to obtain food rewards. Additional training notwithstanding, contextual stimuli within the operant chamber still may have served as cues to evoke memories of previous experiences in the chamber. Hence, future studies that are designed to cross-characterize rats in cognitively complex tasks should consider counterbalancing the order of behavioral assays and should account for the possibility that environmental cues (i.e., position of the operant chamber relative to others, noise, etc.) present during learning of one task may in fact influence learning in a subsequent task.

Considerations for future research

Surprisingly, there were significant vendor differences in performance on the RDT and the PR assay, as well in other ancillary behavioral measures, such as locomotor activity. On the RDT, Charles River rats preferred the large, risky reward to a greater extent than Envigo rats. Rats from Charles River also displayed greater motivation to work for food on the PR assay relative to those from Envigo. Across multiple behavioral tasks (e.g., RDT, Probability Discounting Task), Charles River rats displayed significantly greater locomotor activity compared with locomotor activity of Envigo rats (Table 4). Although these findings were initially unexpected, there is a precedence for vendor differences in rodent behavior in Sprague Dawley and Wistar rat strains. For example, Tsuda et al. (2020) reported that Sprague Dawley rats from Taconic display greater anxiety-like behavior compared with Sprague Dawley rats from Charles River and Envigo. Similarly, Wistar rats from Harlan Laboratories consume more alcohol on an intermittent two-bottle choice task relative to Wistar rats from Charles River and Taconic (Momeni et al., 2015). Variability across suppliers could arise as a result of differences in several factors, including rearing conditions (e.g., number of rats per cage) at the facilities and/or stress of transport from facilities to the research institution (e.g., distance of travel, smells and sounds encountered during transportation, etc.). Random genetic drift, which occurs to a greater extent in outbred rats strains like Long-Evans compared with inbred strains (Eiben & Bomhard, 1999; Gileta et al., 2022; Tsuda et al., 2020), is another potential culprit for such vendor differences in

behavior. Indeed, a recent comprehensive genome-wide association study found substantial genetic divergence between Sprague Dawley rats from Harlan and those from Charles River (Gileta et al., 2022). Such an investigation has yet to be conducted in the Long-Evans strain but given the vendor differences observed in the current study, this is an important next step to gain a better appreciation of genetic contributions to risk taking and reward-related behavior. Regardless of genetic diversity across suppliers, these findings have important implications for ensuring data reproducibility. Furthermore, commercial vendor should be carefully considered when designing experiments to investigate cognitive and neural mechanisms of risk taking as they relate to neuropsychiatric disease. For example, because rats from Charles River display greater risk taking compared with those from Envigo, these rats might be ideal subjects in studies investigating vulnerabilities to the development of neuropsychiatric diseases associated with elevated risk taking (e.g., substance use disorders). Conversely, greater risk aversion in Envigo rats may better lend itself to studies examining factors that may predispose individuals to disorders associated with elevated risk aversion (e.g., eating disorders, anxiety disorders). Hence, like age, sex or housing condition, commercial vendor is another factor that should be controlled for and considered when designing behavioral experiments.

To assess working memory capacity, rats were tested in the Delayed Response Working Memory Task, which required a rat to maintain information about the location of the lever across increasing delays to receive food reinforcement. Unlike other working memory tasks, however, the Delayed Response Working Memory Task may only capture some aspects of working memory, such as short-term memory, and not other equally important aspects, such as active and dynamic manipulation of stored information (Dudchenko, 2004). Indeed, delayed match-to-sample tasks similar to the one used in the current study have been used in humans and animals to specifically assess short-term memory (Barth et al., 1995; Chelonis et al., 2000; Grilly, 1975; Oscar-Berman & Bonner, 1985). Delayed *non-match-to-sample* tasks have been developed and used in rodents to incorporate the "working" aspect of working memory (i.e., active manipulation of stored information). It is therefore worth considering whether a similar relationship between risk taking and working memory capacity would also exist if working memory was assessed with a non-match-to-sample task. The use of a delay non-match-to-sample task, in conjunction with the behavioral assays used to assess flexibility and inhibition, would also more closely represent factors of established executive function models that have been used to probe how executive processes contribute to complex cognitive behavior (Miyake et al., 2000). Future work will thus extend the current findings by incorporating other potentially more representative models of working memory to understand the cognitive basis of risk taking.

Conclusions and implications

The findings presented in the current study expand upon previously established sex differences in risk taking, showing that they are not mediated by differences in motivation to work for food or shock sensitivity, and reveal additional sex differences in cognitive flexibility. More importantly, the results also reveal sex differences in the cognitive mechanisms that may contribute to risky decision making, which could account for sex-specific risk taking phenotypes. Greater working memory capacity in females may contribute to their phenotypic risk aversion by allowing for better recall of recent aversive outcomes. In contrast, the ability to flexibly adapt to changing contingencies (i.e., cognitive flexibility) may influence risk-taking behavior in males. In addition to shedding light on the cognitive substrates underlying risk taking, these findings provide invaluable information about the cognitive endophenotypes that may confer vulnerability to the development of neuropsychiatric diseases, such as substance use disorder and eating disorders. With this information in hand, it may soon be possible to identify vulnerable populations and intervene with prophylactic treatment (e.g., cognitive training) to deter disease development.

Declarations

Conflict of Interest We have no known conflicts of interest.

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