Sex Differences in Risk-based Decision Making

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Highlights

- Male rats made more risky choices in the probability discounting task than females.
- Gonadectomy (GDX) did not influence risk-based decision making in both sexes.
- A high dose of testosterone reduced risky choices in GDX male rats.
- A high dose of amphetamine enhanced risky choices in intact male and female rats.
- 17β-estradiol did not affect risk-based decision making in male or female rats.

Abstract

Decision-making is a complex process essential to daily adaptation in many species. Risk is an inherent aspect of decision-making and it is influenced by gonadal hormones. Testosterone and 17β-estradiol may modulate decision making and impact the mesocorticolimbic dopamine pathway. Here, we explored sex differences, the effect of gonadal hormones and the dopamine agonist amphetamine on risk-based decision making. Intact or gonadectomised (GDX) male and female rats underwent to a probabilistic discounting task. High and low doses of testosterone propionate (1.0 or 0.2 mg) and 17β-estradiol benzoate (0.3 µg) were administered to assess acute effects on risk-based decision making. After 3-days of washout period, intact and GDX rats received high or low (0.5 or 0.125 mg/kg) doses of amphetamine and re-tested in the probabilistic discounting task. Results showed that males made more risk-based choices during the probability discounting task than female rats, but GDX did not influence risky choices. The high dose, but not the low dose, of testosterone reduced risky decision making in GDX male rats. However, 17β-estradiol had no significant effect on risk-based decision making regardless GDX status in either sex. Lastly, the high dose of amphetamine enhanced risky decision making in both intact males and females, with no effect in GDX rats. These findings demonstrated sex differences in risk-based decision making, with males making more risky choices. GDX status influenced the effects of amphetamine, suggesting different dopaminergic regulation in risk-based choices among males and females.

Keywords:

amphetamine, decision-making, operant, gonadal hormones, 17β-estradiol, testosterone, cognition

Introduction:

We routinely face situations that require decisions entailing evaluation of varying costs and benefits associated with different actions. Among the different types of costs that may diminish the subjective value of larger or more preferred rewards, risk/reward decisions involve choices between options that have a lower risk but yield relatively inferior rewards, or more valuable rewards associated with some uncertainty as to whether they will be received (Wiehler and Peters, 2015). Dopamine plays an integral role in modulating cost/benefit decision making as pharmacological blockade of dopamine receptors reduced preference for larger rewards associated with various costs, including effort, delays and uncertainty (Denk et al., 2005; St. Onge et al., 2010; Nunes et al., 2010; Yohn et al., 2015).

The mesolimbic dopamine system is modulated by both sex hormones and sex (Becker, 2009; Morris et al., 2015; Yoest et al., 2014). Studies in humans have reported sex differences in different forms of risk-related decision making, which in turn may be driven by sex hormones (Op de Macks et al., 2016; Peper et al., 2013). Human males and females with high testosterone showed more risk-related behaviour (Stanton et al., 2011) and high testosterone has been linked to poorer decision making in the Iowa gambling task (Reavis and Overman, 2001; van Honk et al., 2004). In addition, increased 17β-estradiol levels may be related to high self-reported risk-taking behaviour in females (Bröder and Hohmann, 2003; Sukolová and Sermány-Schuller, 2011). Thus, testosterone and 17β-estradiol modulate risk-taking behaviours in both human males and females (Op de Macks et al., 2016; Vermeersch et al., 2008a, b).

Preclinical studies in rats have also identified sex differences in risk preferences that may be related to sex hormones. For example, compared to females, male rats show a greater

preference for larger rewards associated with a foot shock delivered in a probabilistic manner (Orsini et al., 2016). Likewise, treatment with supraphysiological doses of chronic testosterone in male rats further increases preference for larger rewards associated with punishment (Cooper et al., 2014). On the other hand, using a probabilistic discounting task wherein rats chose between small/certain and larger/uncertain rewards (in the absence of any punishment), treatment with supraphysiological doses of chronic testosterone in adolescent male rats reduced risky choice (Wallin et al., 2015). This highlights that androgenic modulation of decision making can vary depending on the type of risk an animal faces (punishment or omission of reward) but it is not yet known whether 17β-estradiol or acute physiological doses of testosterone can also modulate risk-based decision making in males.

As alluded to above, sex differences in risk-related decision making may be mediated in part though hormonal modulation of dopamine activity. This may include alterations in dopaminergic tone and axon density within the nucleus accumbens and medial prefrontal cortex (Aubele and Kritzer, 2011; Dazzi et al., 2007; Saigusa et al., 1997), which play a key role in guiding risk/reward decision biases (St. Onge et al. 2010; St. Onge et al., 2012; Stopper et al., 2013; Jenni et al., 2017). 17β-Estradiol increases binding on D_2 dopamine receptors, and facilitates the excitability of dopaminergic transmission in females (Thompson and Moss, 1994; Becker and Hu, 2008). Testosterone increases dopamine synthesis (Purves-Tyson et al., 2012), the expression of D_2 receptor mRNA in the midbrain, and reduced extracellular dopamine levels in the prefrontal cortex after gonadectomy in males (Aubele and Kritzer, 2011; Kritzer et al., 1999; Purves-Tyson et al., 2014). Thus, sex hormones modulate dopaminergic function in both males and females, which may in turn influence risk/reward decision biases (Becker et al., 2012; Sotomayor-Zarate et al., 2014; Walker et al., 2017).

Although some studies have examined how exogenous testosterone may influence risk/reward decision making, considerably less is known about how reductions in testosterone may alter these functions. There is also a lack of data on how estrogens may influence these types of decisions and given that testosterone can be converted to 17β-estradiol, it is important to determine effects of estrogens on decision making in males. In addition, increased preferences for risky rewards in males versus females have been reported when larger rewards may be associated with punishment (Mitchell et al., 2011; Orsini et al., 2015; Simon et al., 2009), which may be related to sex differences in fear expression (Colon et al., 2018; Farrell et al., 2013; Gruene et al., 2015). Yet, how sex may influence decisions guided solely by reward uncertainty in the absence of potential punishment is unknown. To address these issues, we explore sex differences, the effects of gonadectomy, and influence of testosterone or 17β-estradiol, on performance within a probabilistic discounting task, where rats chose between small/certain and larger/uncertain rewards. Additionally, we sought to determine whether gonadal hormone status may interact with manipulations of dopamine transmission in regulating risky choice, using acute treatment with amphetamine, which disrupts adjustments in choice in response to changes in reward probabilities (St. Onge et al., 2010). Here, we hypothesized that males would show greater preference for larger/risky rewards that would be heightened by testosterone and that gonadal hormone status would mediate the risky decisions made in male and female rats by enhancing the response to amphetamine.

Methods:

Animals

Twenty male and sixteen female Long Evans rats (Charles River Laboratories, Montreal, Canada) weighing 275-300 g and 200-250g respectively, at the beginning of behavioural training

were used for the experiment. On arrival, rats were given 1 week to acclimatize to the colony and food restricted to 85-90% of their free-feeding weight for an additional 1 week before behavioural training. Rats were given ad libitum access to water for the duration of the experiment. Feeding occurred in the rats' home cages at the end of the experimental day, and body weights were monitored daily to ensure a steady weight loss during food restriction and maintenance or weight gain for the rest of the experiment. All testing was in accordance with the Canadian Council of Animal Care and were approved by the Animal Care Committee of the University of British Columbia.

Apparatus

Behavioral testing for all experiments was conducted in 12 operant chambers for the males and 4 operant chambers for the females (30.5 × 24 × 21 cm; Med-Associates, St Albans, VT) enclosed in sound-attenuating boxes in different rooms. The boxes were equipped with a fan to provide ventilation and to mask extraneous noise. Each chamber has 2 retractable levers, one located on each side of a central food receptacle where food reinforcement (45 mg; Bioserv, Frenchtown, NJ) was delivered via a pellet dispenser. The chambers were illuminated by a single 100-mA houselight located in the top center of the opposite wall to the levers. Locomotor activity was calculated by the number of photobeam breaks that occurred during a session as four infrared photobeams were mounted on the sides of each chamber. The chambers were connected to a computer though a digital interface that recorded all experimental data.

Experimental timeline

The experimental timeline is shown in Figure 1. Each animal received Lever-Pressing Training 1 week after the initiation of food restriction. After 5-6 days of Lever-Pressing Training, animals were trained on the probabilistic discounting task to examine the baseline choice performance. After completion of the baseline training, half of the animals (equal sexes) were gonadectomised and half were given a sham surgery (described below). Animals were given 1 week for recovery, and then were retrained on the task to examine the baseline choice performance after the surgery. On the following day, rats received vehicle treatment and then the next day the first dose of hormone injections and the discounting test 3h, 24 and 48 h later to test rapid and delayed effects of the steroid hormones. Rats then were re-trained to baseline choice performance for 3-7 days to ensure baseline performance and then received a second dose of hormone and then a discounting test. The same procedure was repeated for the third dose of hormone injections. After completion of hormone treatment, rats received two doses of amphetamine and examined their choice performance. Vehicle treatments were always conducted 24h prior to hormone or drug treatments (Figure 1).

Lever-Pressing Training

Our initial training protocols have been described previously (Stopper et al., 2013). Briefly, rats were first trained under a fixed-ratio 1 schedule to a criterion of 60 presses in 30 min, first for one lever, and then repeated for the other lever (counterbalanced left/right between subjects). Rats were then trained on a simplified version of the full task - 90 trial sessions beginning with the levers retracted and the operant chamber in darkness, and each trial requiring them to press one of the two levers within 10s of its insertion for reinforcement of a single pellet delivered with 50% probability. If the rat did not respond to a lever within 10s after the lever was

presented, the chamber was reset to the intertrial state until the next trial (omission). This procedure was used to familiarize the rats with the association of lever pressing and food delivery, as well as the probabilistic nature of the full task. In every pair of trials, the left or right lever was presented once, and the order within the pair of trials was randomized. Rats were trained for approximately 5-6 days to reach a criterion of less than 10 omissions out of 90 trials.

Probability Discounting Task

The task was modified from Cardinal and Howes' (2005) procedure, which has been previously used to assess the role of dopaminergic and noradrenergic neurotransmission in risk-based decision making (St Onge and Floresco, 2009; Montes et al., 2015). Rats received daily sessions 6-7 days per week, consisting of 72 trials each day, separated into 4 blocks of 18 trials over 48min, where the probability of receiving a large reward decreased each block (100, 50, 25, 12.5%). A session began in darkness with both levers retracted (the intertrial state). A trial began every 40s with insertion of one or both levers into the chamber 3s after the illumination of the houselight (the format of a single trial is shown in Fig. 1). One lever was assigned as the Large/Risky lever, the other the Small/Certain lever. The combination of levers with the amount of reward given remained consistent throughout training but counterbalanced the position (left or right). If the rat did not respond by pressing a lever within 10s of lever presentation, both levers were retracted and the house light was turned off without any reward was given (omission). When a lever was chosen, both levers retracted. Pressing the Small/Certain lever always delivered one pellet with 100% probability, but pressing the Large/Risky lever delivered 4 pellets with 0.5 s apart but with a specific probability of the trial block. When food was delivered, the houselight remained on for another 4s after a response was made, which was followed by reversion of the chamber to the intertrial state. The 4 blocks were comprised of 18 trials with 8

forced-choice trials followed by 10 free-choice trials. During a forced-choice trial, only one lever was presented (4 trials for each lever, randomized in pairs) allowing animals to learn the amount of food associated with each lever pressing and the respective probability of receiving reward over each block. During a free-choice trial, both levers were presented and the rat chose either the Small/Certain or the Large/Risky lever. The probability of receiving large reinforcement after pressing the Large/Risky lever was initially 100%, then 50%, 25%, and 12.5%, respectively, for each successive block. For each session and trial block, the probability of receiving the large reinforcement was drawn from a set probability distribution. Therefore, on any given day, the probabilities in each block may slightly vary from the assigned probability, but on average across many training days, the actual probability experienced by the rat will approximate the set value. Using these probabilities, selection of the Large/Risky lever would be more advantageous in the first 2 blocks (100% and 50% probability trial), and more disadvantageous in the last block (12.5%) compared to selection of the Small/Certain lever, whereas rats could obtain an equivalent number of food pellets after responding on either lever during the 25% block. Therefore, in the last 3 trial blocks (50%, 25% and 12.5%) of this task, selection of the larger reward option carried with it an inherent ‘‘risk’’ of not obtaining any reward on a given trial. Latencies to initiate a choice and overall locomotor activity (photobeam breaks) were also recorded. The criteria for the completion of training on the task was that they chose the Large/Risky lever during the first trial block (100% probability) on at least 80% of successful trials.

Surgery

Surgery was performed under isoflurane anesthesia (5% in oxygen during induction, 3% in oxygen during maintenance). Treatment groups were assigned according to their baseline

choice performance over the last 3 days of training such that there was no difference between groups in all blocks.

Males were either bilaterally castrated (GDX) or received sham-castrations. For castrations, both testes were extracted through a small incision made at the posterior tip of the scrotum and were ligated with a monofilament suture. Sham operations involved incisions into the skin and muscle layers of the scrotum that were sutured without removing the testes. *Females* were either bilaterally ovariectomized (GDX) or received sham-ovariectomy. For ovariectomy, both ovaries were extracted through a small horizontal incision in the abdominal wall directly over each ovary and were ligated with a monofilament suture. Sham operations involved incisions through the skin and abdominal muscle that were sutured without removing the ovaries. Prior to surgery each rat was given a subcutaneous injection of Ketoprofen (5 mg/kg body mass) as an analgesic and immediately afterward Flamazine cream (1% silver sulfadiazine) was applied to the incision. All rats received Ketoprofen 24 and 48 hours after surgery at 5 mg/kg subcutaneously as an analgesic. After surgery, rats were singly housed and one week was allowed for full recovery from surgery prior to further experimentation.

Drug Treatments

Both GDX and sham *males* were assessed for changes in baseline choice performance following surgery. Upon returning to baseline performance, all rats received vehicle or hormone treatment, as described previously on the experimental timeline. Between each hormone or drug treatment, baseline performance was also re-assessed. Single injections of testosterone proprionate (0.2mg or 1mg, s.c.) and 17β-estradiol benzoate (0.3µg, s.c.) were used to assess their acute activational effects.

Both GDX and sham *females* were assessed for changes in baseline choice performance following surgery. Upon returning to baseline performance, all rats received hormone treatment accordingly to the experimental timeline. A single injection of 17β- estradiol benzoate (0.3µg) were used to assess their acute activational effects.

For both males and females, after completion of hormone treatments, we assessed the effects of amphetamine (0.125mg/kg or 0.5mg/kg i.p.) in both treatment groups (GDX and sham). Each drug test consisted of a two-day sequence. The first day, rats were injected with saline, and the second day, with one of the two doses of amphetamine (counterbalanced). After each injection, rats were placed in their home cages and behavioral testing commenced 10 min later. After the first test sequence, rats were trained for 3 days before receiving another test sequence of saline and the other dose of amphetamine (based on St. Onge and Floresco, 2009). There were no differences in performance across the two saline injection test, so the data from these tests were averaged in the analysis and compared to the two amphetamine challenges.

Data Analyses

The primary dependent measure of interest was the proportion of choices directed toward the large reward lever for each block of free-choice trials, factoring out trial omissions. For each block, this was calculated by dividing the number of choices of the large reward lever by the total number of successful trials (ie, those where the rat made a choice). Data from each vehicle treatment test day were averaged. The effect of trial block was always significant ($p<0.001$) for the probabilistic discounting task and will not be discussed further. Mixed model analyses of variance were conducted with between-subject factors of sex (male, female) and surgery (sham, GDX) and within-subjects factors of block (12.5, 25, 50, 100% of probability of the large/risky

reward), hormone dose (testosterone 0,0.2,1 mg or 17β- estradiol benzoate 0, 0.3 µg), time (pre-GDX, post-GDX) or amphetamine dose (0,0.125 0.5 mg/kg) were conducted using Statstica (v. 13, StatSoft, Tulsa, OK, USA). Post-hoc tests utilized Newman-Keuls comparisons whereas a priori comparisons used a Bonferroni correction. Effect sizes are given as partial η_p^2 or Cohen's *d* where appropriate. The significance level was set at $\alpha = 0.05$. Response latencies and the number of trial omissions were analyzed with one-way repeated-measures ANOVAs.

Whenever a significant main effect of a treatment or group on probabilistic discounting was observed, we conducted a supplementary analysis to further clarify whether changes in choice biases were due to alterations in sensitivity to reward (win-stay performance) or negative-feedback (lose-shift performance) (Stopper and Floresco, 2014). Animals' choices during the task were analyzed according to the outcome of each preceding trial (reward or non-reward) and expressed as a ratio. The proportion of win-stay trials was calculated from the number of times a rat chose the large/risky lever after choosing the risky option on the preceding trial and obtaining the large reward (a win), divided by the total number of free-choice trials where the rat obtained the larger reward. Conversely, lose-shift performance was calculated from the number of times a rat shifted choice to the small/certain lever after choosing the risky option on the preceding trial and was not rewarded (a loss), divided by the total number of free- choice trials resulting in a loss. This analysis was conducted for all trials across the four blocks. We could not conduct a block X block analysis of these data because there were many instances where rats either did not select the large/risky lever or did not obtain the large reward at all during the latter blocks. Changes in win-stay performance were used as an index of reward sensitivity, whereas changes in lose-shift performance served as an index of negative-feedback sensitivity (Stopper et al. 2013). These data were analyzed with multifactorial ANOVAs with Response type (win

stay/lose shift) as one within-subject factor, drug/hormone treatment as another within-subject factor (when applicable) and Sex and Gonadectomy status as a between-subjects factor.

Results:

Males showed greater bias for large/risky rewards versus females, that was not altered by gonadectomy

Figure 2 shows the proportion of risky choices made by male and female rats on the last 3 days of training pre-surgery (baseline, Fig. 2A) and for 3 days of training 2 weeks post-surgery (Fig. 2B). Analysis of the choice data at baseline revealed a sex X trial block interaction ($F(3, 93)=3.5$, $p=0.018$; $\eta_p^2=0.10$; Fig 2A). Males showed significantly greater choice of the large/risky lever in both the 25% ($p<0.02$, Cohen's $d= 0.563$) and 12.5% ($p<0.00018$, Cohen's $d=0.645$) probability trial blocks compared to females. There were no other significant main effects or interactions.

Gonadectomy (GDX, short-term – 2 weeks post-surgery) did not influence performance in this task compared to sham animals (all p's> 0.55. Figure 2B). As previous studies have shown differences in performance between short versus long-term GDX (Bimonte-Nelson et al., 2003; Aubele and Kritzer, 2011), we conducted a further analysis to determine whether there were any surgery effects 6 weeks after GDX. However, even 6 weeks after GDX, there were no significant effects of GDX relative to sham (all p's >0.51. Figure 2 C), and males still made more choices on the risky lever in the 25% (Cohen's $d= 0.90$) and 12.5% (Cohen's d= 0.85) probabilities than females regardless of GDX (p's <0.05) status; sex by block interaction: ($F(3, 93)=5.463$, $p<0.0017$, $\eta_p^2=0.15$).

The lower levels of risky choice observed in female rats under baseline conditions and post GDX appeared to be associated with an increased tendency to shift to the small/certain lever after a non-rewarded risky choice (lose-shift behavior). Although analysis of these data yielded a response type X sex interaction that only approached statistical significance ($F(1,32)=3.36$, $p=0.076$), an exploratory comparison confirmed that females displayed higher lose-shift ratios than males ($F(1,34)=4.28$, $p=0.046$ Fig 2D). There were no other interactions across pre/post surgery phases or GDX status (all p's>0.25). Thus, female rats were more sensitive to the ability of non-rewarded risky choices to influence subsequent choices away from the risky option compared to males.

At baseline females displayed a greater number of trial omissions (1.063 ± 0.3) than males (0.246 ± 0.1) main effect of sex $F(1, 27)=4.929$, $p=0.035$, $\eta_p^2= 0.15$) but there was no significant effect of GDX on the number of trial omissions (all p's > 0.35). Females had significantly greater choice latency compared to males (main effect of sex $F(1, 27)=4.886$, $p=0.036$, $\eta_p^2= 0.15$), and there was a main effect of time ($F(1, 27)=4.848$, $p=0.036$, $\eta_p^2= 0.15$) showing choice latency to be significantly greater with time, following either GDX or sham surgery. As expected, females also showed significantly greater locomotor activity compared to males at baseline (main effect of sex $F(1, 29)=20.409$, $p=0.00001$, $\eta_p^2= 0.41$, while surgery had no significant effect on locomotor activity (all p's > 0.11) (see Table 1).

High, but not low, Testosterone treatment reduced risky decision making in the GDX males only

Figure 3 shows the acute effects of low (0.2 mg/kg) and high (1.0 mg/kg) dose of testosterone on risk-based decisions in sham (3A) and GDX (3B) male rats. The high, but not low, dose of testosterone (1 mg) reduced proportion of risky choices in the lowest probability block (12.5%;

p<0.002, Cohen's *d*=0.815) compared within the GDX males but not in the sham males (p=0.40; dose X block X surgery F(6, 90)=2.025, p=0.07, η_p^2=0.12). The high dose of testosterone increased the probability of a lever press for the 25% block (p<0.04) but this is no longer significant after a Bonferroni correction. There was a main effect of blocks as expected (F(6, 45)=22.119, p<0.0001, η_p^2=0.596) but no other significant main or interaction effects (p's> 0.11).

There was no significant effect of testosterone treatment (at low or high dose) on the number of trial omissions or choice latency (all p's > 0.07; Table 1). Figure 3C and D shows the risky choices at 24 h after treatment with low and high dose of testosterone. As it can be seen, no significant effect on the risk-based choices were observed after testosterone administration (main effect of dose: p=0.571, main effect of block F(3, 54)=32.802, p<0.0001), all other main or interaction effects p's>0.22). However, both the low (Cohen's *d*= 0.45) and high (Cohen's *d*= 0.73) doses of testosterone treatment decreased locomotor activity after 24h of testosterone treatment (p <0.035; Table 1). There was also a main effect of surgery, where GDX males have significantly reduced locomotor activity than sham males (F(1, 14)=9.246, p<0.009, η_p^2= 0.40) (see Table 1).

17β-estradiol treatment did not influence risky decision making in males or females

Figure 4 shows the effect of 17β-estradiol benzoate on the risky choices across different groups. Figure 4A and 4B partition the data based on GDX status collapsed across sexes and panels C-F partition these data further by sex. There were no significant effects of 17β-estradiol treatment on risky decision making in either sex (no significant main or interaction effects with sex (all p's >0.17). There was a significant interaction of 17β-estradiol dose X surgery X block effect (F(6,

180)=2.412, p=0.029, η_p^2=0.074), but post-hoc tests failed to find anything meaningful. There was a significant main effect of block (F(3, 90)=70.774, p<0.0001, η_p^2=0.702) but no other main or interaction effects (all p's >0.17). After 24 hrs of 17β-estradiol treatment, no changes were observed in the risky choices neither in male nor female rats independently of GDX status. There was also no significant effect of 17β-estradiol treatment on the number of omissions in either males or females (all p's >0.39) (Table 1). However, 17β-estradiol treatment significantly increased choice latency in females 24h after treatment (F(1, 29)=7.829, p=0.009, η_p^2=0.21) (table 1). There were no other significant effects on choice latency (all p's >0.15) (Table 1).

Amphetamine increased risky choice in both males and females, but this effect was blunted by GDX.

Figure 5 shows the effect of amphetamine (AMPH) on risky choices across the different groups and sexes. One female sham rat displayed a marked increase in omissions over all trial blocks, and its choice data were excluded from the analysis. Analysis of these data yielded a significant AMPH dose X block interaction (F (6,186)=5.805, p<0.001) and a dose X block X GDX interaction (F (6,186)=3.15, p<0.01), but no other 3 or 4 way-interactions with the Sex Factor (all p's>0.10). Figure 5A and B partition the data based on GDX status, collapsed across sexes and panels C-F partition these data further by sex. Simple main effects revealed that in sham animals, the 0.5 mg/kg dose increased risky choice in the 25% and 12.5% probability blocks (p<0.05), whereas the 0.125 mg/kg dose only increased risky choice in the 12.5% block (p<0.05). In GDX rats, this effect was markedly blunted, wherein the 0.5 mg/kg dose of amphetamine did not induce a statistically significant increase in risky choice during any block (all p's>0.15). AMPH treatment also did not disrupt the inherent sex difference (block X sex interaction (F (3,93)= 8.71, p<0.0001, η_p^2=0.22). These findings replicate previous finding

where AMPH can increase risky choice under these conditions (St. Onge and Floresco, 2009; St. Onge et al., 2010), and further show that this effect is comparable in both males and females, and that indicate that GDX in both sexes attenuates the ability of amphetamine to alter risky choice.

The increase in risky choice induced by AMPH across all groups was driven by a reduction in lose-shift tendencies. Analysis of these data revealed a significant dose X response type interaction ($F(2,26)= 3.57$, $p=0.034$) but no other interactions by dose with the sex or GDX factors (all p's>0.10). Simple main effects analysis further confirmed that AMPH treatments did not alter win-stay behavior ($p>0.30$), but the 0.5 mg/kg dose significantly reduced lose-shift behavior ($p<0.001$; Fig 5G). There was also a significant response type X sex interaction ($F(1,31)=4.66$, $p=0.048$) indicating that across all drug doses and conditions, females displayed greater lose-shift tendencies (0.27 +/-0.03) vs males (0.19+/-0.02) but no differences in win-stay behavior (females=0.91+/-0.02; males 0.94 +/-0.01).

With respect to other performance measures, the 0.5 mg/kg dose of AMPH increased the number of omissions (dose X sex X surgery interaction $F(3, 93)=8.851$, $p=0.006$, $\eta_p^2=0.22$), wherein sham females had a greater number of omissions than all other treatment groups despite the interaction (all p's< 0.0002). In contrast, the 0.125 mg/kg dose dose of AMPH decreased the number of omissions (main effect of treatment $F(1, 29)=5.520$, $p=0.026$, $\eta_p^2=0.16$). The low dose of AMPH decreased choice latency in females (treatment by sex interaction $F(1, 30)=8.900$, $p=0.006$, $\eta_p^2=0.23$) but females still showed greater choice latency than males (all p's< 0.02). The high dose of AMPH produced a greater choice latency in females (main effect of sex ($F(1, 28)=20.429$, $p=0.0001$, $\eta_p^2=0.42$). The high dose of AMPH tended to induced a longer latency in sham females in comparison to female GDX (sex by surgery interaction ($F(1, 28)=3.797$, $p=0.06$, $\eta_p^2=0.12$). A low dose of AMPH produced no significant effect on locomotor activity, although

there was a trend ($F(1, 23)=3.728$, $p=0.066$, $\eta_p^2=0.14$). As expected, high dose AMPH increased locomotor activity in all treatment groups (day by sex interaction ($F(1, 25)=16.770$, $p=0.0004$, $\eta_p^2=0.40$), and increased locomotor activity in females more than males ($p < 0.005$) (Table 2).

Discussion:

The present study revealed notable sex differences during risk/reward decision making, in that male rats made more risky choices on a probabilistic discounting task compared to females. However, these sex differences did not appear to be driven by adult levels of gonadal hormones, as gonadectomy did not alter risky choice in either sex, nor did they alter sex differences. Acute treatment with a higher dose of testosterone propionate, reduced risky choice in GDX, but not intact, male rats. Furthermore, 17β-estradiol benzoate had no significant effect on decision making of either males and females regardless of their gonadectomy status. Lastly, amphetamine increased risky decision making of both male and female rats in a dose-dependent manner, and this effect of amphetamine was more pronounced in rats with intact gonads compared to GDX animals.

Male rats made more risky choices during probabilistic discounting task than female and gonadectomy did not influence their risk-related decision making.

Under baseline conditions, male rats made more risky choices when the probability of obtaining the food reward was relatively low (25% or 12.5%) compared to female rats. Importantly, there were no sex differences in choice of the large/risky reward when the probability of obtaining a larger food reward was 50 or 100%, suggesting that the lower levers of risky choice observed in females was unlikely due to reduced preference for larger rewards. These results are consistent with another a study using another type of risky decision making

task in which the risk of larger reward was associated with a negative consequence of footshocks (Orsini et al., 2016). On the other hand, Georgiou et al. (2018) used a rat version of the Iowa Gambling Task (IGT) which required subjects are four choices with probability of 90%, 80%, 60% or 50% to receive 1, 2, 3 or 4 pellets of reward respectively, where the punishment was a 'time out' in the form of not receiving a reward for 10, 20, 30 or 40 seconds respectively before the next trial began. Over the course of learning, female rats tended to show a bias towards the most advantageous choice faster than males, whereas once both groups reached asymptotic levels of performance, male rats chose the most advantageous choice more often than females. With respect to present study, females showed the lower levels of risky choice in the latter trial blocks when the risky option had lower utility compared to the small/certain option. This suggest that under these conditions female rats are more likely to avoid risky choices after non-rewarded choices after pressing the risky lever, even though pressing the risky lever was neither advantageous nor disadvantageous. Thus, it is plausible that females in the present study were more capable of distinguishing the advantageous lever during lower probability 12.5% trial block, in comparison to males. This sex difference appeared to be driven in part by an increased tendency by females to shift their choice after encountering an occasional loss regardless of whether it was in the long-term advantageous or disadvantageous, compared to males, suggesting that females may be more loss averse. This tendency is also observed in human studies evaluating risk/loss aversion using the Risky-Gains Task (Lee et al., 2009) and the Cambridge Gambling Task (van den Bos et al., 2014).

Sex differences have also been observed along different types of decision-making in rodent models. Adult female mice display less impulsive behavior than males by reducing their preference for a larger, delay reward in intertemporal decision-making (Koot et al., 2009; Perry

et al., 2007). Along similar lines, sex differences have been proposed in effortful in decision-making, as females may exert more effortful control than males (Bjorklund and Kipp, 1996), but may also show a reduced preference for larger rewards associated with a greater effort cost (Uban et al., 2012). Our results here add evidence of sex differences in decision making that encompass risky choices, and that males made more risk-based choices in comparison to females when animals are choosing between certain and uncertain rewards without encountering potential punishment.

Gonadectomy has been shown to influence some aspects in decision-making in males (Tan and Vyas, 2016; Wallin-Miller et al., 2017) and females (Uban et al., 2012). However, in the present study, gonadectomy did not exert changes in risky choices in either males or females. This discrepancy can be due the different types of costs that were associated with larger rewards across different tasks (Tan and Vyas, 2016; Uban et al., 2012), or the rewards used (Wallin-Miller et al., 2017), as in the later study risky choices were elicited by ethanol, and this stimulus could have a stronger impact on motivation and on the mesolimbic reward pathway. The idea that sex-hormone regulation of decision making can differ depending on the type of cost associated with larger/preferred rewards is further bolstered by the findings of studies examining how stress affects these processes. Specifically, acute stress can influence certain forms of cost/benefit decision making without affecting others, in that it can reduce preference for larger rewards associated with a greater effort cost, but not those that are delayed or delivered in a probabilistic manner (Shafiei et al., 2012; Bryce and Floresco, 2016; Bryce et al., 2020). The present data further highlight that the hormonal and neurochemical modulation of different forms of cost/benefit decision making can vary based on the type of cost being evaluated.

Testosterone and estradiol effects on risk/reward decision making- interactions with gonadectomy

We found that a high dose of testosterone decreased the risky choice during the 12.5% probability trial block compared to vehicle-treated GDX rats. This is partially consistent with a previous study using a similar probability discounting task with chronic testosterone throughout adolescence (5-14 weeks old) (Wallin et al., 2015). In that study they found that very high levels of testosterone (7.5 mg) reduced risky choice compared to vehicle treated intact rats during 50%, 25% and 0% probability trial blocks, whereas the present study found a significant effect of lower physiological doses of testosterone only during the 12.5% probability trial block. This inconsistency can be due to the gonadal status of the rats, as well as dose, age, and the differences between puberty or in adulthood when testosterone was administered. In the present study, we used an acute physiological dose of testosterone in adult rats and found that our high physiological dose reduced risky choices. Intriguingly, studies with supraphysiological doses of testosterone using the punishment (foot shocks) discounting task (Cooper et al., 2014), effort discounting (Wallin et al., 2015) or delay discounting task (Wood et al., 2013) showed that these large doses of chronic testosterone increased choice of large reward option. This further highlights that increases in testosterone activity can differentially affect decisions involving choice between larger and smaller rewards in a manner that is critically dependent on the type of cost associated with these rewards. Thus, testosterone reduces the impact of punishments, delays or effort costs have on diminishing the subjective value of larger rewards, resulting in an increase preference for these rewards. Conversely, increased testosterone activity appears to increase sensitivity to reward uncertainty, reducing choice of larger/uncertain rewards.

Androgen receptors (ARs) are located throughout the mesolimbic regions and on dopaminergic neurons in the prefrontal cortex (PFC) and dorsolateral striatum (Tobiansky et al., 2018), as these areas are reported as critical brain regions involved during risk/reward decision making (Orsini et al., 2015; Worthy et al., 2016). Indeed, systemic testosterone, but not 17β-estradiol, decreased the basal dopamine levels in the PFC in male rats (Aubele and Kritzer, 2011) and burst firing in the VTA is androgen, but not 17β-estradiol, sensitive (Locklear et al., 2017). This is important as testosterone can be converted to 17β-estradiol via aromatase and thus can modulate dopaminergic systems via it's actions on AR or estrogen receptors (ER). Furthermore, systemic administration of an aromatase inhibitor (letrozole) decreased dopaminergic turnover in the PFC in both male and female rats (Kokras et al., 2018). Thus, androgens exert an important role in attenuating the disruptions in cortical innervations of dopamine induced by gonadectomy in male rats (Kitzer, 2000). Our observation that treatment with testosterone but not 17β-estradiol alters risk/reward decision making in males on risky decision making add additional support to the notion that that testosterone may alter these processes via AR but not ER and may do so by modulating mesocorticolimbic DA activity system.

In contrast to the above mentioned findings, we found no significant effect of ovariectomy or 17β-estradiol (0.3μg) on risk-based decision making in female rats. These findings are consistent with evidence that estrous cycle does not influence risk-based decision making where risky choice is coupled with footshoock (Orsini et al., 2016) or in the IGT (Georgiu et al., 2018). Conversely, a previous report showed that OVX rats made more effort for a lager reward, an effect that is not observed in intact animals and is reversed by estradiol (Uban et al., 2012), suggesting that estradiol may influence some aspects of decision making involving effort costs, but not those involving reward uncertainty.

Interestingly, previous studies have found that 17β-estradiol (5 μg or 10 μg) can potentiate the release of dopamine in the dorsal striatum with amphetamine (Song et al., 2019), and in the nucleus accumbens (NAc) with cocaine (Tobiansky et al., 2016). Yet, 17β-estradiol treatment did not alter risky choice, which can also be altered by pharmacological increases in dopamine transmission (St. Onge et al., 2009). Alternatively, 17β-estradiol may be able to augment drug-induced dopamine release and related behaviors, but on its own, may be insufficient to alter behaviourally evoked changes in dopamine (St. Onge et al, 2012). On the other hand, the fact that we observed that amphetamine increased risky choice in both sexes but only in intact and not GDX rats, suggests that ovarian hormones can influence the ability of amphetamine to modulate risk/reward decision making. Collectively these studies suggest that although a low dose of 17β-estradiol may not directly influence risky choice, it may interact with the effects of dopaminergic drugs to alter reward-related decision biases. Supporting this, 17β-estradiol enhanced the amphetamine-induced place preference (Silverman and Koenig, 2007) and OVX decreased quinpirole specific binding in the NAc, which is reversed by 17β-estradiol administration (Le Saux et al., 2006).

Amphetamine enhanced risky choice making in both male and female rats, and this effect of amphetamine was stronger in sham rats compared to gonadectomised rats.

Consistent with previous reports (St. Onge and Floresco, 2009; Floresco and Whelan, 2009; St. Onge et al., 2010), amphetamine treatments increased risky choice, and this effect was comparable across male and female rats. This general effect was associated with a reduction in negative feedback sensitivity, as rats were less likely to shift choices after a non-rewarded risky choice. This amphetamine-induced increase of risky decision during the probability discounting task is likely driven by activation of D_1 and D_2 receptors in different terminal regions (St. Onge

and Floresco, 2009; St. Onge et al., 2011; Stopper et al., 2013; Pes et al., 2017). However, the ability of amphetamine to alter risky choices was not observed in GDX rats relative to those with intact gonads. The enhancing effect of gonadal hormones on amphetamine-induced risky decision making is likely due to modulation of different dopaminergic systems between the two sexes. Indeed, cocaine-induced increase of dopamine levels in the dorsolateral striatum is enhanced by 17β-estradiol in female rats but not in males (Cummings et al., 2014). On the other hand, castration has been reported to enhance amphetamine-mediated dopamine release (Hernandez et al., 1994). In this regard, it should be noted that although we did not find any statistical interactions between sex and gonadectomy status on the effects of amphetamine, visual inspection of the data suggests that this blunting effect of gonadectomy was more pronounced in females vs males. Thus, even though our experiment design was insufficient to detect differences between these groups, it is possible that compared to males, female gonadal hormones may exert a more permissive influence on the ability of stimulant drugs to alter risk/reward decision making.

Conclusion:

Sex differences exist in the cognitive manifestations of disorders involving the mesolimibic dopamine pathway such as schizophrenia, impulsive disorders and Parkinson's disease, with males showing greater cognitive disruption. In the present study, we found that females were less likely to choose the riskier options than males, which was associated with an increase sensitivity to losses. Surprisingly gonadectomy did not influence risky decision making in either sex under baseline conditions but did alter the manner in which amphetamine can influence action selection. These results suggest that dopaminergic regulation of risk related behavior might be different between the sexes.

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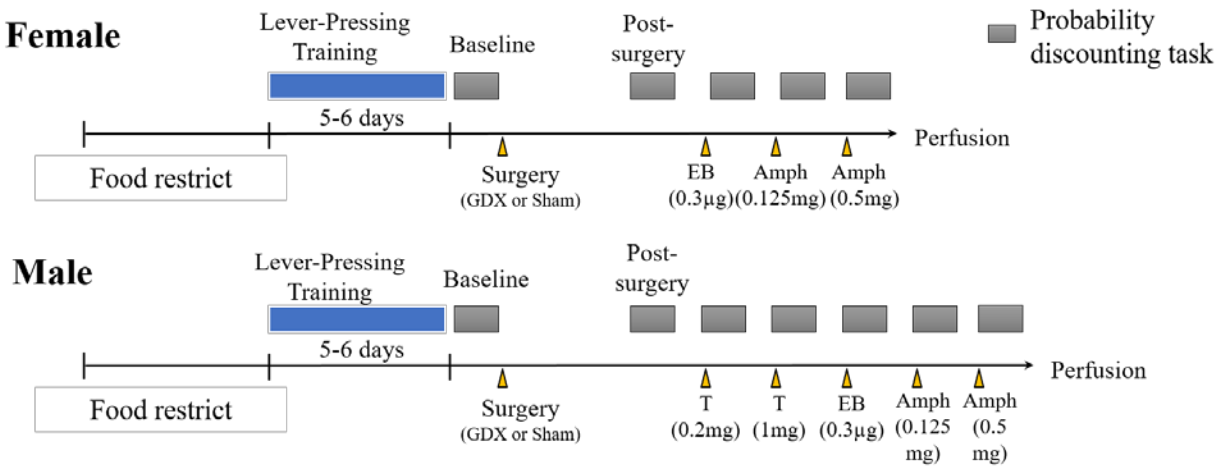

Figure 1. Experimental timeline. GDX=Gonadectomy, EB= 17β-estradiol benzoate, Amph= Amphetamine, T= Testosterone

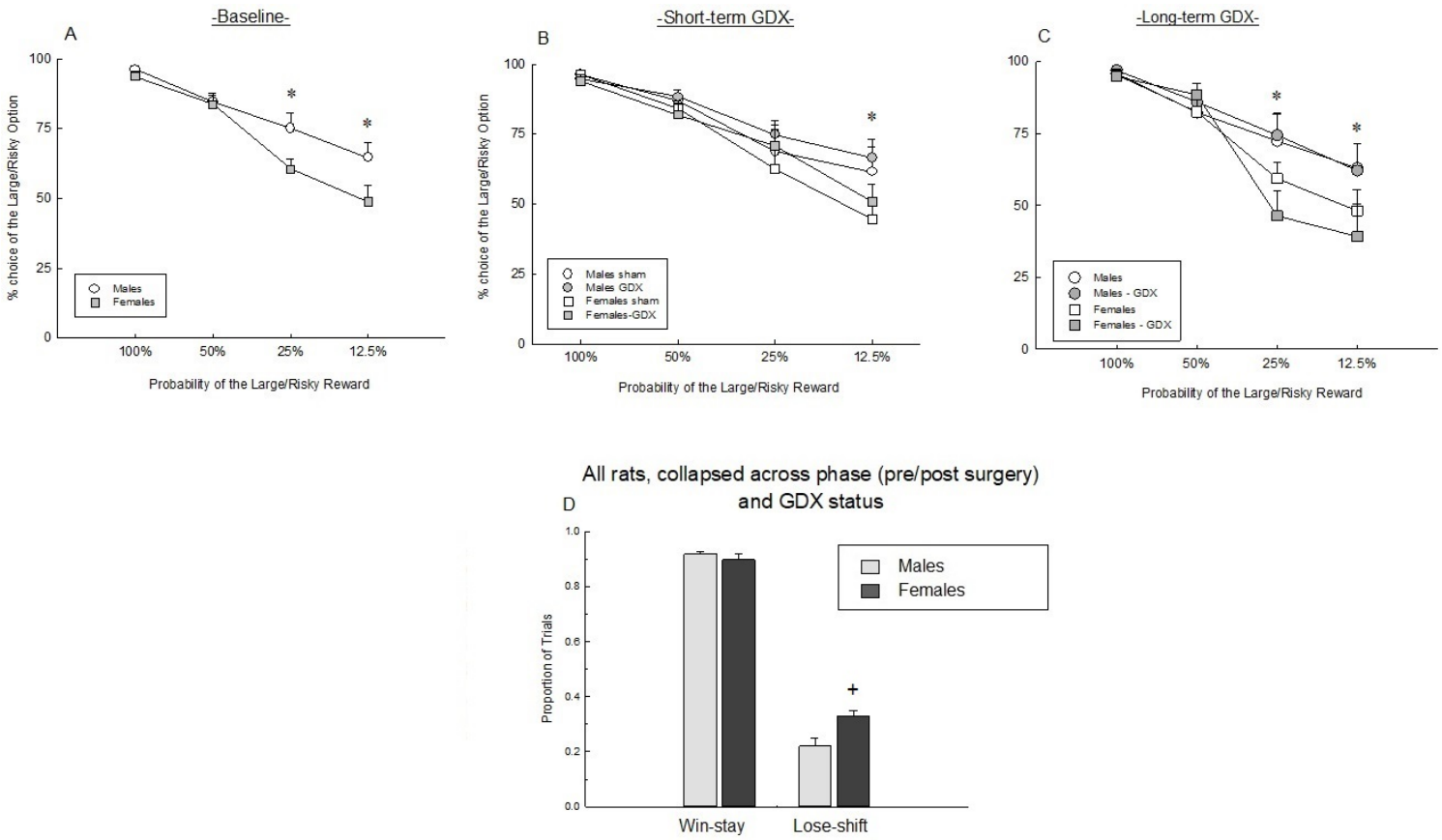

Figure 2. Group means (+ SEM) of the percentage choice of the Large/Risky lever during 100%, 50%, 25% and 12.5% trial blocks at baseline (2A) short-GDX (2B) and long-term GDX (2C) in male and female rats. Panel D shows the Win-stay and Lose-shift ratio during the probabilistic discounting task. n= 19 males, 16, females at baseline; n= 8 -10 per group in short and long-term GDX.
* denotes $p<0.05$ vs females at 25% and 12.5% block regardless of GDX;
+ denotes $p<0.05$ vs males. Abbreviations: GDX, gonadectomy.

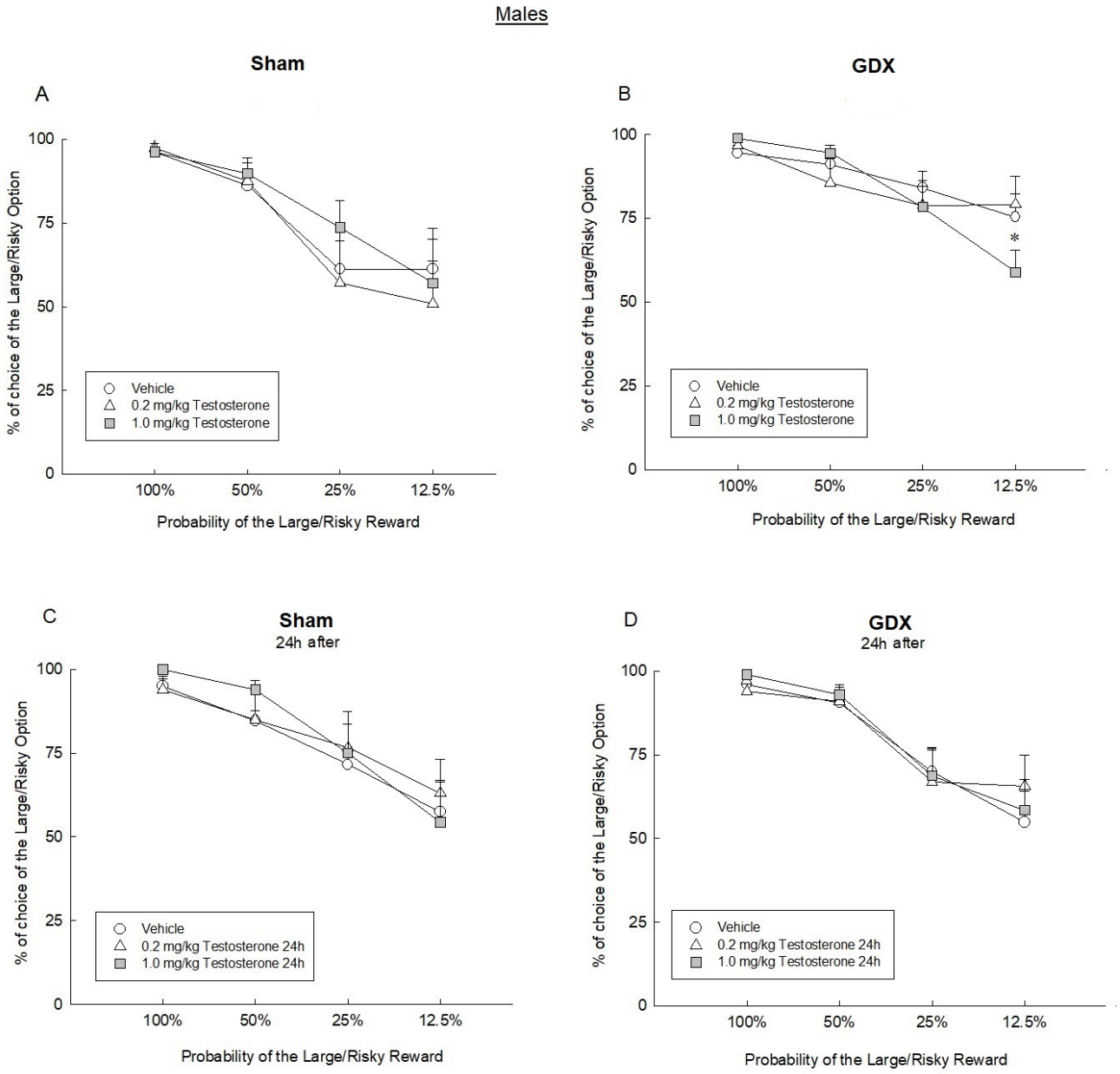

Figure 3. Group means (+ SEM) of the percentage choice of the Large/Risky lever during 100%, 50%, 25% and 12.5% trial blocks in both, males sham (2A) and GDX (2B) treated with low and high dose of testosterone, and 24 hours after testosterone administration in sham (2C) and GDX (2D) rats. n= 8-10 per group. * denotes $p<0.05$ vs vehicle. Abbreviations: GDX, gonadectomy

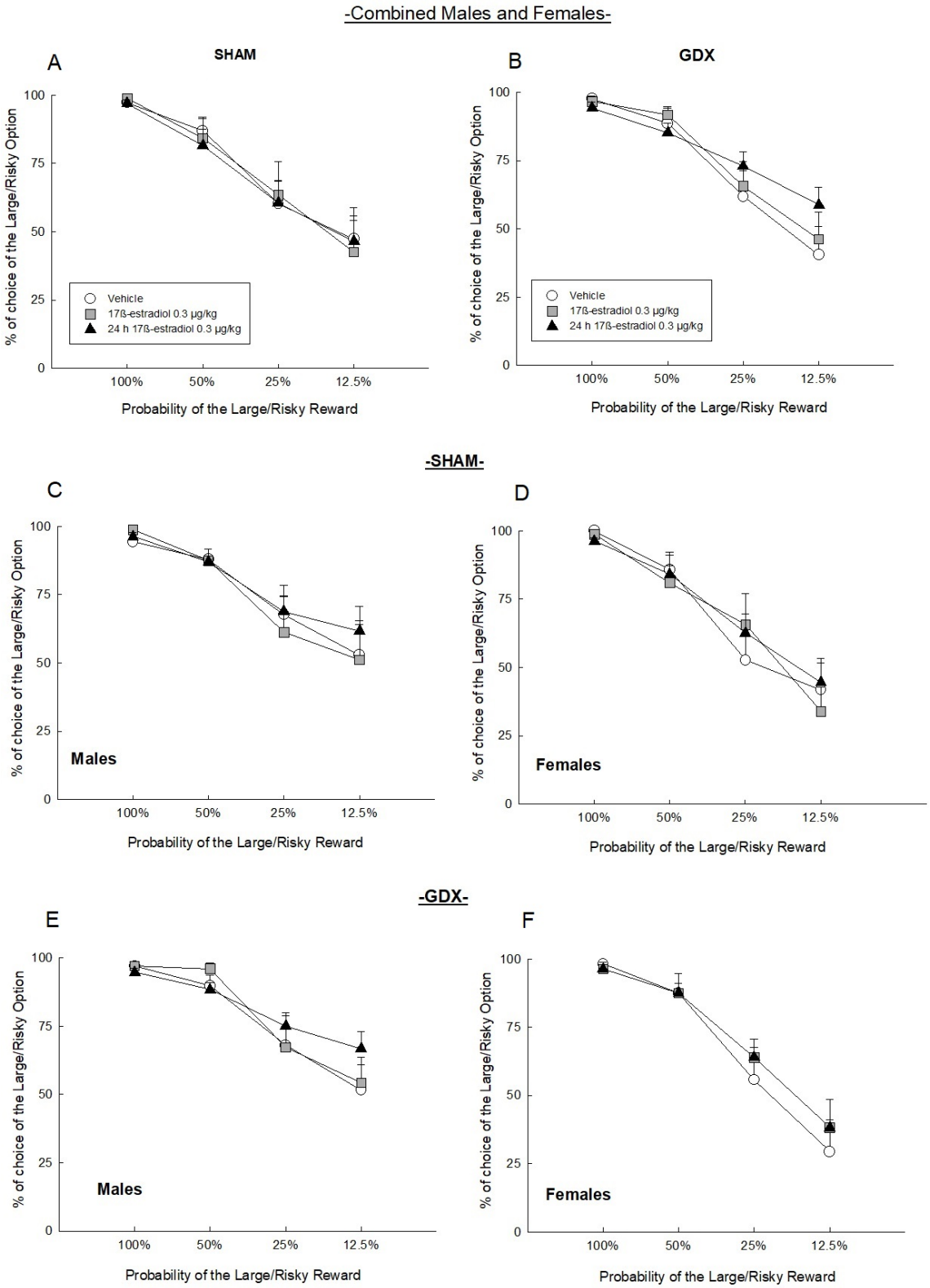

Figure 4. Group means (+ SEM) of the percentage choice of the Large/Risky lever during 100%, 50%, 25% and 12.5% trial blocks in combined males and females sham (5A) and GDX (5B) rats, males sham only (5C) and female sham only (5D), treated with 17β-estradiol and 24 hours after 17β-estradiol treatment. Abbreviations: GDX, gonadectomy.

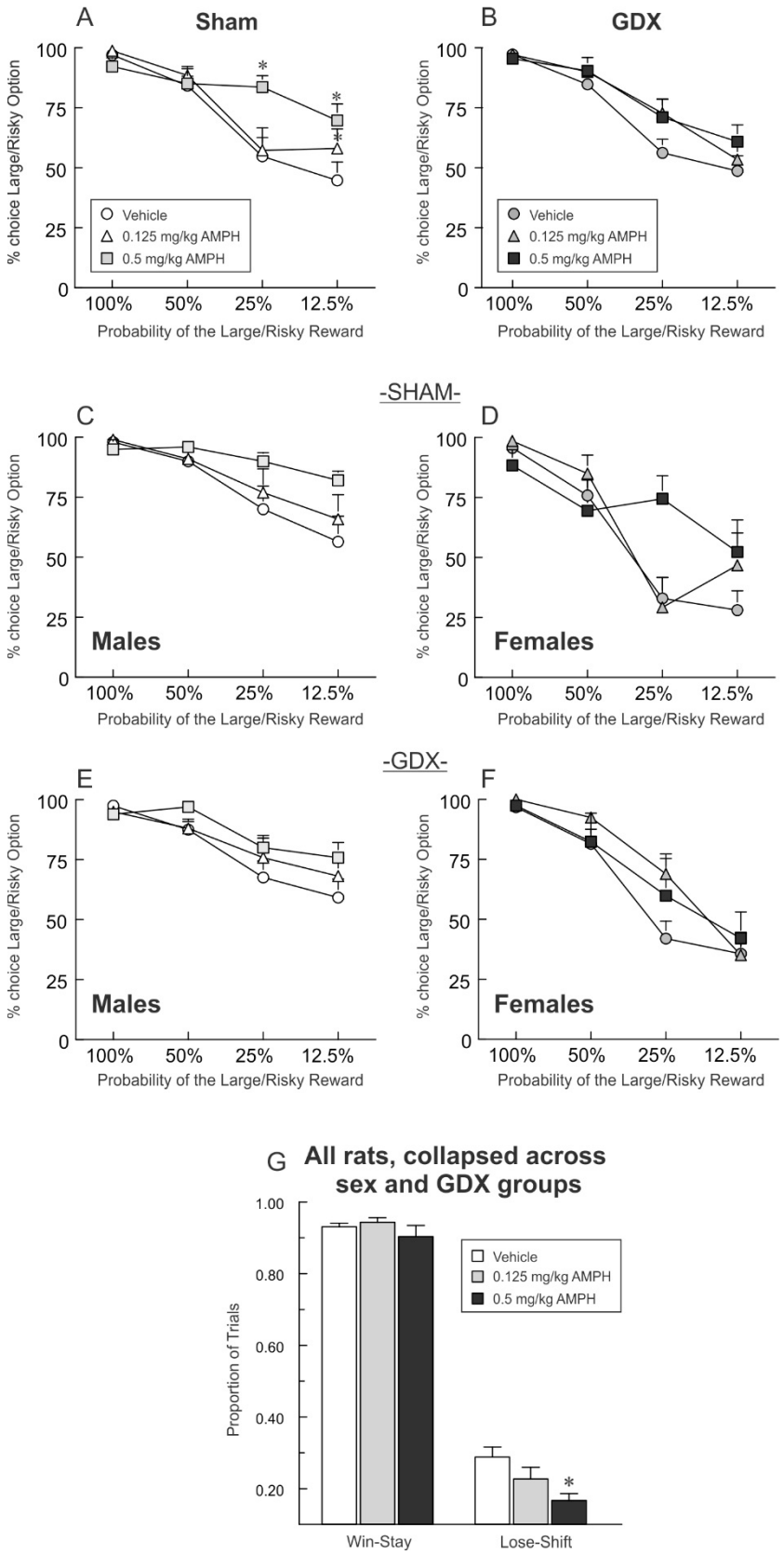

Figure 5. Group means (+ SEM) of the percentage choice of the Large/Risky lever during 100%, 50%, 25% and 12.5% trial blocks in combined males and females sham (5A) and GDX (5B) rats, males sham only (5C), females sham only (5D), male GDX only (5E), female GDX only (5F) treated with a low (0.125 mg/kg,) or high (0.5 mg/kg) dose of amphetamine. Panel E shows the Win-stay and Lose-shift ratio during the probabilistic discounting task. n= 8-10 per group. * denotes $p<0.05$ vs vehicle. Abbreviations: GDX, gonadectomy, AMPH, amphetamine.

Table 1. Effect of hormonal treatments on omissions, latency to decision-making and locomotor activity of sham and GDX males and females rats.

	Group	*Omissions*	*Latency*	*Locomotor activity*
Males sham	Baseline	0.246 ± 0.098	0.836 ± 0.111	1389.6 ± 83.8
	Vehicle	0.111 ± 0.111	0.754 ± 0.149	1761.0 ± 220.3
	Testosterone 0.2 mg	0.500 ± 0.341	0.808 ± 0.154	1662.5 ± 180.4
	Testosterone 1 mg	0.200 ± 0.133	0.782 ± 0.142	1723.8 ± 179.5
	17β-estradiol 0.3 µg	0.777 ± 0.521	0.923 ± 0.216	1649.3 ± 136.7
	Vehicle – 24 hrs	0.555 ± 0.338	0.826 ± 0.185	1895.4 ± 148.9
	Testosterone 0.2 mg – 24 hr	1.600 ± 0.921	0.960 ± 0.224	1516.6 ± 164.9[a]
	Testosterone 1 mg – 24 hr	1.900 ± 0.983	1.018 ± 0.218	1509.8 ± 117.1[a]
	17β-estradiol 0.3 µg – 24 hrs	0.778 ± 0.434	0.830 ± 0.195	1653.7 ± 147.5
Males GDX	Short tem	0.111 ± 0.078	0.877 ± 0.142	1263.7 ± 122.5
	Long term	0.777 ± 0.662	0.772 ± 0.149	1651.7 ± 171.6
	Vehicle	0.333 ± 0.235	0.762 ± 0.141	1244.3 ± 80.2
	Testosterone 0.2 mg	0.222 ± 0.222	0.842 ± 0.144	1211.7 ± 107.4[aa]
	Testosterone 1 mg	0.000 ± 0.000	0.808 ± 0.153	1206.9 ± 158.3[aa]
	17β-estradiol 0.3 µg	1.111 ± 1.111	0.965 ± 0.226	1059.9 ± 132.2
	Vehicle – 24 hrs	0.333 ± 0.236	0.811 ± 0.113	1309.5 ± 210.7
	Testosterone 0.2 mg – 24 hr	0.000 ± 0.000	0.824 ± 0.146	1158.9 ± 131.7 [aa]
	Testosterone 1 mg – 24 hr	0.000 ± 0.000	0.790 ± 0.118	1010.9 ± 126.9 [aa]
	17β-estradiol 0.3 µg – 24 hrs	0.778 ± 0.662	1.032 ± 0.192	990.3 ± 135.4

	Group	*Omissions*	*Latency*	*Locomotor activity*
Females Sham	Baseline	1.063 ± 0.266[b]	1.146 ± 0.109[c]	2101.2 ± 174.1[d]
	Vehicle	2.285 ± 0.993	1.532 ± 0.199	1714.0 ± 223.6
	17β-estradiol 0.3 µg	3.714 ± 1.085	1.622 ± 0.156	1712.9 ± 244.0
	Vehicle 17β-estradiol – 24 hrs	4.429 ± 1.270	1.766 ± 0.198	1765.1 ± 217.4
	17β-estradiol 0.3 µg – 24 hrs	2.857 ± 1.280	1.985 ± 0.181[e]	1626.4 ± 160.1
Females GDX	Short-Term GDX	1.000 ± 0.600	1.244 ± 0.208	1562.3 ± 101.4
	Long-Term GDX	4.1667 ± 1.973	1.749 ± 0.302	1486.6 ± 102.3
	Vehicle	2.000 ± 1.017	1.234 ± 0.217	1439.1 ± 192.6
	17β-estradiol 0.3 µg	1.750 ± 0.818	1.333 ± 0.177	1423.5 ± 169.9
	Vehicle – 24 hrs	2.250 ± 1.048	1.435 ± 0.211	1443.0 ± 154.9
	17β-estradiol 0.3 µg – 24 hrs	1.500 ± 0.567	1.525 ± 0.213[e]	1513.0 ± 136.3

Table 1. Mean ± SEM of omissions, latency and locomotor activity of male and female rats.

[a] denotes $p<0.05$ testosterone – 24h vs vehicle (reduced locomotor activity after 24h of testosterone treatment)

[aa] denotes $p<0.01$ GDX vs sham (GDX males had reduced locomotor activity than sham males treated with testosterone)

[b] denotes $p<0.05$ females vs males (females had grater omissions than males)

[c] denotes $p<0.05$ females vs males (females had greater latency than males)

[d] denotes $p<0.001$ females vs males (females had grater locomotor activity than males)

[e] denotes $p<0.05$ 17β-estradiol – 24h vs vehicle (increased latency after 24h of 17β-estradiol treatment)

Table 2. Effect of low and high dose of amphetamine on omissions, latency to decision-making and locomotor activity of sham and GDX males and females rats.

	Group	*Omissions*	*Latency*	*Locomotor activity*
Males Sham	Vehicle AMPH 0.125 mg/kg	0.111 ± 0.111	0.797 ± 0.180	1128.5 ± 117.0
	AMPH 0.125 mg/kg	0.000 ± 0.000[a]	0.835 ± 0.231	1622.3 ± 189.3
	Vehicle AMPH 0.5 mg/kg	0.222 ± 0.222	0.677 ± 0.174	1764.8 ± 136.1
	AMPH 0.5 mg/kg	0.000 ± 0.000[a]	0.823 ± 0.202	2184.7 ± 278.6[b]
Males GDX	Vehicle AMPH 0.125 mg/kg	0.777 ± 0.662	0.772 ± 0.149	1651.7 ± 171.6
	AMPH 0.125 mg/kg	0.555 ± 0.338[c]	0.800 ± 0.137	1039.9 ± 92.3
	Vehicle AMPH 0.5 mg/kg	0.555 ± 0.555	0.796 ± 0.156	1138. 4 ± 100.9
	AMPH 0.5 mg/kg	1.222 ± 0.722	0.948 ± 0.122	1804.5 ± 288.7[b]
Females Sham	Vehicle AMPH 0.125 mg/kg	5.333 ± 2.962	2.599 ± 0.755	1405.3 ± 276.6
	AMPH 0.125 mg/kg	6.000 ± 5.507	1.780 ± 0.587[e]	1372.0 ± 152.5
	Vehicle AMPH 0.5 mg/kg	1.667 ± 0.333	2.044 ± 0.178	1531.7 ± 140.9
	AMPH 0.5 mg/kg	8.000 ± 4.933[d]	2.244 ± 0.990[f]	2345.3 ± 377.7[b,g]
Female GDX	Vehicle AMPH 0.125 mg/kg	4.1667 ± 1.973	1.749 ± 0.302	1486.6 ± 102.3
	AMPH 0.125 mg/kg	1.000 ± 0.683[c]	1.468 ± 0.332[e]	1322.2 ± 193.5
	Vehicle AMPH 0.5 mg/kg	3.000 ± 1.633	1.616 ± 0.304	1386.3 ± 217.9
	AMPH 0.5 mg/kg	1.500 ± 1.310	1.499 ± 0.274[f]	2376.5 ± 237.9[b,g]

Table 2. Mean ± SEM of omissions, latency and locomotor activity of male and female rats.

[a] denotes $p<0.05$ AMPH 0.125 and 0.5 mg/kg vs vehicle (reduced omissions after AMPH treatment)

[b] denotes $p<0.0005$ AMPH 0.5 mg/kg vs vehicle (increased locomotor activity by AMPH)

[c] denotes $p<0.05$ AMPH 0.125 mg/kg vs vehicle (reduced omissions after AMPH treatment)

[d] denotes $p<0.05$ AMPH 0.5 mg/kg vs vehicle, (increased omissions after AMPH treatment)

[e] denotes $p<0.05$ AMPH 0.125 mg/kg vs vehicle (reduced latency after AMPH treatment)

[f] denotes $p<0.0005$ females vs males, (greater latency in females than in males after AMPH 0.5 mg/kg)

[g] denotes $p<0.005$ females vs males (greater locomotor activity in females than in males treated with AMPH 0.5 mg/kg)