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# anabolic-androgenic steroids and decision making: probability and effort discounting in male rats

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## Abstract

Anabolic-androgenic steroid (AAS) abuse is implicated in maladaptive behaviors such as increased aggression and risk taking. Impaired judgment due to changes in the mesocorticolimbic dopamine system may contribute to these behavioral changes. While AAS are known to influence dopamine function in mesocorticolimbic circuitry, the effects on decision making are unknown. This was the focus of the present study. Adolescent male Long-Evans rats were treated chronically with high-dose testosterone (7.5 mg/kg) or vehicle (13% cyclodextrin in water), and tested for cost/benefit decision making in two discounting paradigms. Rats chose between a small reward (1 sugar pellet) and a large discounted reward (3 or 4 pellets). Probability discounting (PD) measures sensitivity to reward uncertainty by decreasing the probability (100, 75, 50, 25, 0%) of receiving the large reward in successive blocks of each daily session. Effort discounting (ED) measures sensitivity to a work cost by increasing the lever presses required to earn the large reward (1, 2, 5, 10, 15 presses). In PD, testosterone-treated rats selected the large/uncertain reward significantly less than vehicle-treated controls. However, during ED, testosterone-treated rats selected the large/high effort reward significantly more than controls. These studies show that testosterone has divergent effects on different aspects of decision making. Specifically, testosterone increases aversion to uncertainty but decreases sensitivity to the output of effort for reward. These results have implications for understanding maladaptive behavioral changes in human AAS users.



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Contributors: KGW designed the study, carried out behavioral testing, collected and analyzed the data, and wrote the manuscript. Jasmin Alves carried out behavioral testing and collected and analyzed the data. RIW advised throughout the study and edited the manuscript. All authors have approved the final manuscript.

**Keywords**

anabolic agents; testosterone; decision making; discounting; operant behavior; food reward

## 1. Introduction

Anabolic-androgenic steroids (AAS) are drugs of abuse used by athletes to increase muscle mass and enhance athletic performance. While media attention focuses on steroid use among elite athletes, the use of AAS is far more widespread. As many as 4 million Americans have used AAS (Pope et al, 2013). AAS are in high schools, fitness centers, and "rejuvenation" clinics. A typical AAS user is a young man in his late teens or early 20s (Pope et al, 2014). Among U.S. high school students, 4-6% of boys have used AAS (Johnston et al, 2013), comparable to the rates of crack cocaine or heroin use. It is estimated that AAS use among men in their 20's is even higher (Pope et al, 2013).

Emerging evidence highlights a range of adverse health effects from chronic AAS abuse, including cardiovascular, hepatic, reproductive and psychiatric dysfunction (Pope et al, 2014). Indeed, as many one third of AAS users meet DSM criteria for psychoactive substance dependence (Pope et al, 2013). Furthermore, AAS users have higher mortality rates than the general population, often due to suicide or homicide (Thiblin et al, 2000). In this regard, AAS use is associated both with depression and anxiety (Perry et al, 1990), as well as increased aggression, commonly known as "'roid rage" (Hall et al, 2005). Thus, a key danger of AAS abuse reflects the likelihood that users will engage in behaviors that pose risks to themselves and to those around them. In a study of American high-school students, AAS use was associated with risky sex, drinking and driving, carrying a weapon, and not wearing a helmet or seat belt (Middleman *et al*, 1995). Psychological evaluations of human users have also implicated AAS in impaired decision making stemming from feelings of invincibility (Pope and Katz, 1990). To evaluate decision-making ability under the influence of AAS, the present study tested cost/benefit tasks of probability and effort in male rats treated chronically with high-dose testosterone beginning in adolescence. Cost/benefit decision making depends on prefrontal cortical (PFC)-striatal circuitry (Floresco *et al*, 2008a), that develops during adolescence (Blakemore and Robbins, 2012). AAS perturb dopamine (DA) function in this system (Kindlundh *et al*, 2001; Kurling-Kailanto *et al*, 2010; Wood *et al*, 2013), and have the strongest behavioral effects when introduced in adolescence (Salas-Ramirez *et al*, 2008). Therefore, chronic high-dose testosterone exposure beginning in adolescence has potential to alter decision making behavior.

To test decision making, discounting paradigms require subjects to choose between two rewards: a small "safe" reward, and a large reward that is "discounted" or made less desirable by pairing with a cost such as delay, effort, uncertainty, or punishment. In a test of punishment discounting, we have demonstrated that testosterone-treated rats are more likely than controls to choose a large reward paired with a footshock over a small reward with no shock (Cooper *et al*, 2014). Similarly, testosterone-treated rats are more willing to wait for a large/delayed reward compared to vehicle-treated controls (Wood *et al*, 2013). The present study investigated the effects of chronic high-dose testosterone on response to uncertainty (probability discounting; PD) and physical effort (effort discounting; ED). In PD, rats chose

between a small/certain reward and a large/uncertain reward delivered with decreasing probability. Endogenous testosterone levels in humans correlate with increased risk taking under uncertainty in both the Iowa Gambling Task (Stanton *et al*, 2011) and the stock market (Coates and Herbert, 2008). Therefore, we hypothesized that AAS would increase risk taking during PD, by increasing selection of the large/uncertain food reward. ED tests a subject's willingness to exert physical effort to obtain reward. In ED, rats choose between a small/low effort reward and a large/high effort reward. This ED paradigm is relevant to human AAS users, as body builders and athletes expend tremendous physical effort in pursuit of their aesthetic and athletic goals. Thus, we expected that testosterone-treated rats would be willing to work harder to obtain food reward, reflecting decreased sensitivity to effort.

## 2. Methods

### 2.1 Animals

Male Long-Evans rats (5 weeks of age at the start, Charles River Laboratories, MA) were pair-housed under a reversed 14L:10D photoperiod. 42 rats were treated with testosterone or vehicle, and were trained and tested for PD (vehicle: n=12; testosterone: n=11) or ED (vehicle: n=10; testosterone: n=9). All behavior was tested during the first 4 hours of the dark phase. To approximate AAS use by humans, rats remained gonad-intact. As in our previous studies (Cooper *et al*, 2014), rats were food-restricted to maintain a slow rate of growth (3-4 g/day) and facilitate operant responding. Body weights in testosterone- and vehicle-treated rats did not differ at the start of the study (vehicle: 139.2±1.6 g, testosterone: 138.5±1.4 g) or throughout behavioral training and testing. Experimental procedures were approved by USC's Institutional Animal Care and Use Committee and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, 8$^{th}$ Ed (National Research Council, National Academies Press, Washington DC; 2011).

### 2.2 AAS treatment

Beginning at least 2 weeks prior to behavioral training, rats received daily sc injections of testosterone (7.5 mg/kg; Steraloids, RI) or aqueous vehicle [3% ethanol and 13% cyclodextrin (RBI, MA)] 5 d/week. Testosterone is the prototypical AAS, and is the most common performance-enhancing substance (55.5%) detected in urine tests by World Anti-Doping Agency-accredited laboratories (WADA, 2012). The 7.5 mg/kg dose is equivalent to the doses used by humans to enhance performance, and has previously been used to test the effects of AAS on decision making and cognition in rats (Cooper et al, 2014; Wood *et al*, 2013; Wallin and Wood, 2015). Daily injections were administered early in the dark phase, immediately prior to behavioral training and testing. Importantly, although testosterone treatment was initiated during adolescence at 5 weeks of age (Spear, 2000), behavioral testing was not complete until rats had reached young adulthood, at least 14 weeks of age.

### 2.3 Operant Chambers

Testing was conducted in operant chambers (Med Associates, VT) with 2 retractable levers flanking a pellet dispenser and food cup (Figure 1). Chambers were illuminated by a house light and were enclosed in sound-attenuating boxes with fans for ventilation.

## 2.4 Pre-training

**2.4.1 Lever Training—**Initially, rats were trained to respond on each lever to receive 45mg sucrose pellets (Bio-Serv Inc., Frenchtown, NJ). Next, rats were habituated to lever insertion. Each 20-second trial began in darkness with both levers retracted in the inter-trial interval (ITI) state. Three seconds later, the house-light was illuminated and 1 lever was inserted into the chamber. Left and right levers were each inserted once per pair of trials in random order. If the rat responded within 10 seconds, 1 pellet was delivered and the house-light stayed on for 4 seconds before returning to ITI. If the rat failed to respond within 10 seconds, the chamber reverted to ITI and the trial counted as an omission. Training continued until rats omitted <5 of 80 trials.

**2.4.2 Reward discrimination—**These sessions consisted of 80 trials divided into 5 blocks. Each block consisted of 8 forced-choice trials and 8 free-choice trials. In the forced-choice trials, 1 lever was inserted into the chamber on each trial (4 trials/lever). In free-choice trials, both levers were inserted, and the rat could select either the small or large reward lever. No probability or effort costs were imposed during reward discrimination. A response on the small reward lever delivered 1 pellet; a response on the large reward lever delivered 4 or 3 pellets in PD and ED respectively (Figure 1). Location of the small and large reward levers (left vs. right) was counterbalanced among rats. Rats were required to complete 80 trials with >80% selection of the large reward lever.

## 2.5 Testing

**2.5.1 General testing procedures—**Behavioral testing took place in daily sessions, 5 days/week. A session consisted of 80 trials, divided into 5 blocks. Each block included 8 forced-choice and 8 free-choice trials. In the forced-choice trials, 1 lever was inserted into the chamber on each trial (4 trials/lever), demonstrating the probability of reward or effort cost associated with each lever in each block. In free-choice trials, both levers were inserted, and the rat could select either the small or large reward lever. Rats had 10 seconds to make a response before the levers retracted and the trial was counted as an omission.

**2.5.2 Probability Discounting—**Procedures were modified from St. Onge and Floresco (2009). Selection of the small/certain reward lever always resulted in delivery of 1 pellet. Selection of the large/uncertain reward lever delivered 4 pellets with decreasing probability on each block (100, 75, 50, 25, and 0%). On rewarded trials, the houselight remained illuminated for 2 seconds after lever selection while pellets were delivered. On unrewarded trials the chamber reverted immediately to ITI after the rat responded on the large/uncertain reward lever.

**2.5.3 Effort Discounting—**The ED paradigm was based on Uban *et al* (2011). Selection of the small/low effort reward lever resulted in delivery of 1 pellet. Selection of the large/high effort reward lever resulted in 3 pellets with an increasing response requirement in each block [fixed ratio (FR) 1, 2, 5, 10, and 15]. After the rat responded once on the large/high effort reward lever, the other lever retracted. If the response requirement was not completed within 15 seconds, the trial was counted as incomplete, no pellets were delivered, and the chamber reverted to ITI. The 3:1 ratio for large and small rewards with ED is derived from

Uban et al (2011), who delivered large and small rewards at a ratio of 4:2. The 3:1 ratio used here was designed to minimize the potential for satiety in ED while maintaining the same difference between large and small rewards (2 pellets). The 3:1 ratio decreases the maximum pellets/ED session to 200, closer to that of PD (110 pellets).

### 2.6 Data Analysis

Vehicle- and testosterone-treated rats were tested for 15 days, until choice behavior stabilized. Stability was assessed as in Cooper et al (2014). Data from the last 5 consecutive days of testing were analyzed by RM-ANOVA with test day as the repeated measure. Choice behavior was considered stable when there was no effect of test day on selection of the large reward at each block. There was no effect of testosterone on task acquisition. Subsequently, data from the last 5 days were averaged for each rat. In both PD and ED, preference for the large discounted reward was determined by the proportion of 8 free-choice trials/block in which each rat selected the large reward lever. Trial omissions were also analyzed. For PD, total pellets won by vehicle- and testosterone-treated rats were also compared. In ED, incomplete trials were expressed as a percentage of trials in which the large reward was selected (excluding block FR1, in which incomplete trials were not possible). As in St. Onge and Floresco (2009), all behavioral measures were averaged for testosterone- and vehicle-treated rats in each block and compared by Repeated Measures (RM)-ANOVA with block as the repeated measure. When there was a main effect of testosterone treatment, post-hoc analysis compared behavior within each block by Dunnett's test (St. Onge and Floresco, 2009).

To investigate how testosterone affected sensitivity to reward delivery and omission in PD, Win-Stay (WS) and Lose-Shift (LS) behavior was analyzed on a trial-by-trial basis. A WS occurred when the rat received the large reward (win), and responded on the large reward lever again in the following trial (stay). A LS occurred when the rat received no pellets from the large reward lever (loss), and selected the small reward on the following trial (shift). WS and LS ratios were computed as the number of times each behavior occurred divided by the total number of wins or losses respectively. WS and LS ratios were averaged for vehicle- and testosterone-treated rats in each probability block and compared by RM-ANOVA. Note that WS ratios could not be calculated for the 0% block, as wins were not possible, and LS ratios could not be calculated for the 100% block, since losses were not possible.

## 3. Results

### 3.1 Probability Discounting

**3.1.1 Selection of large reward lever—**Figure 2A compares selection of the large/uncertain reward lever by testosterone- and vehicle-treated rats during 8 free-choice trials in each probability block. By RM-ANOVA, there was a significant effect of probability block on large lever selection, with all animals decreasing their selection of the large/uncertain reward as reward probability decreased ($F_{4,18}$=446.0, $p<0.05$). Lever selection in vehicle-treated rats was similar to that of vehicle controls in previous studies (St. Onge and Floresco, 2009; Ghods-Sharifi *et al*, 2009). Vehicle-treated rats chose the large reward over 90% of the time in the 100% probability block, and 87.1±2.7% of the time at 50% probability.

However, even when the probability of receiving the large reward was only 25%, vehicle-controls chose the large/uncertain reward in 51.5±6.2% trials. By RM-ANOVA, there was a main effect of drug, with testosterone-treated rats selecting the large/uncertain reward significantly less than vehicle-treated controls ($F_{1,21}$=8.58, p<0.05), reflecting reduced tolerance for uncertainty in the testosterone-treated rats. At each of the three lowest probability blocks (50, 25 and 0%), testosterone-treated rats chose the large/uncertain reward significantly less often than vehicle-treated controls (p<0.05) For example, at the 25% probability block, testosterone-treated rats selected the large reward in only 33.6±5.9% of trials. There was no drug × probability block interaction ($F_{4,18}$=2.70, p>0.05).

**3.1.2 Pellets earned—**Figure 2B shows pellets earned in free-choice trials at each probability block relative to potential earnings by always selecting the large or small reward lever. By RM-ANOVA, there was a significant effect of probability block, with all rats receiving fewer pellets as probability decreased. Although testosterone-treated rats selected the large uncertain reward significantly less often than vehicle-treated controls at the 50, 25, and 0% probability blocks, there was no effect of testosterone on total pellets earned ($F_{1,21}$=1.318, p>0.05), and no drug × probability block interaction ($F_{4,18}$=2.17, p>0.05). As indicated by Figure 2B, the optimal strategy changes throughout the PD session: at 50% probability, selection of the large/uncertain reward lever yields the most pellets. At 0% probability, selection of the small/certain reward lever is most advantageous. At 25% probability, both levers are equally advantageous. As evident from these data, testosterone-treated rats adopted a different behavioral strategy (preference for the small/certain reward lever) (Figure 2A) without significantly decreasing pellets earned (Figure 2B).

**3.1.3 Trial Omissions—**Omitted trials were negligible during free-choice trials. In 5 days of testing with 23 rats, only 8 trials were omitted out of 4600 free-choice trials (<0.5%). During forced-choice trials on the small/certain reward lever, only 1 trial was omitted out of 2300. However, in forced-choice trials on the large/uncertain reward lever, trial omissions were more common (Figure 2C). By RM-ANOVA, there was a significant effect of probability block, with all rats increasing omissions as the probability associated with the large reward decreased ($F_{4,18}$=4.86, p<0.05). There was no effect of drug on trial omissions ($F_{1,21}$=0.001, p>0.05), and no interaction of drug × probability block ($F_{4,18}$=0.24, p>0.05). Thus, rats responded to the decrease in reward probability by omitting forced-choice trials in which they would get no pellets for their response.

**3.1.4 Win-Stay and Lose-Shift behavior—**WS and LS ratios, measuring sensitivity to reward delivery and omission, respectively, are in Figure 3. As in previous studies (Stopper *et al* 2012), vehicle-treated rats are more likely to “stay” after a win than to “shift” after a loss, indicating greater sensitivity to reward delivery than to reward omission. Averaging WS and LS ratios for vehicle-treated rats over all probability blocks, the average WS ratio for vehicle-treated rats was 0.93±0.20 and the average LS ratio was 0.40±0.32. By RM-ANOVA, there was a significant effect of probability block on both WS ($F_{3,19}$=8.54, p<0.05) and LS ratios ($F_{3,19}$=111.19, p<0.05), indicating that rats responded to decreasing probability throughout the session by decreasing WS and increasing LS behavior. While WS ratios did not differ between vehicle- and testosterone-treated rats ($F_{1,21}$=0.85, p>0.05),

testosterone-treated rats trended toward higher LS ratios in each probability block ($F_{1,21}$=3.52, p=0.07). Thus, the decreased preference for the large/uncertain reward lever in testosterone-treated rats (Figure 2A) reflects an increased sensitivity to reward omission, rather than a decreased sensitivity to reward delivery.

## 3.2 Effort Discounting

**3.2.1 Selection of large reward lever—**Figure 4 compares selection of the large reward lever (including complete and incomplete trials) by testosterone- and vehicle-treated rats during 8 free-choice trials in each effort block during reward discrimination training and during ED testing. During both training and testing, rats completed 80 trails/day (40 each, free-choice and forced-choice) where selection of the large reward delivered 3 pellets/trial (max. 200 pellets/day).

However, with reward discrimination training, the response requirement to obtain the large reward remained constant at FR1 across all blocks. As shown in Fig. 4A, both testosterone- and vehicle-treated rats maintained a strong and consistent preference for the large reward lever during training (overall preference: vehicle 96.9±0.8%, testosterone 96.2±1.3%). They obtained 194.8±1.2 pellets/day. Even during the last block of free-choice trials, vehicle controls selected the large reward lever on 7.25 of 8 trials (90.6±3.5%). Responses in testosterone-treated rats were similar (89.1±4.5% of 8 trials, p>0.05 vs controls). Based on these results, neither vehicle-nor testosterone-treated rats show evidence of satiety in 80 trials/day.

However, with ED testing there was a significant effect of effort block on large lever selection by RM-ANOVA. Both groups of rats decreased their selection of the large/high effort reward as the response requirement increased ($F_{4,14}$=8.90, p<0.05). Vehicle- and testosterone-treated rats exhibited a robust preference (>90% of trials) for the large/high effort reward in blocks FR1 through FR5. Selection of the large/high effort reward by vehicle-treated rats decreased rapidly after FR5, reaching 53.2±10.6% of trials by FR15. In testosterone-treated rats, preference for the large/high effort reward decreased more slowly as the response requirement increased, reaching 77.2±6.1% of trials in FR15. There was a main effect of drug on lever preference, with testosterone-treated rats selecting the large/high effort reward significantly more than vehicle-treated controls ($F_{1,17}$=4.96, p<0.05). There was no drug × probability block interaction ($F_{4,14}$=1.78, p>0.05). However, in the last two effort blocks (FR10 and FR15), the increase in preference for the large/high effort reward by testosterone-treated rats approached significance (both p=0.07).

**3.2.2 Incomplete trials—**Incomplete trials were uncommon during forced- and free-choice trials at FR2-FR10: in 5 days of testing with 19 rats, there were only 3 incomplete trials out of 4560 total trials (<0.01%). However, at FR15, incomplete trials increased in vehicle- and testosterone-treated rats during forced- and free-choice trials (Figure 5). By RM-ANOVA with trial type (forced vs. free) as the repeated measure, there was a significant effect of drug ($F_{1,17}$=4.96, p<0.05), with testosterone-treated rats making fewer incomplete trials than vehicle controls. There was no effect of trial type on incomplete trials at FR15, but there was an interaction of trial type × drug ($F_{1,17}$=7.04, p<0.05). During

forced-choice trials, testosterone- and vehicle-treated rats made similar numbers of incomplete trials (1.2±0.5% and 2.3±1.4%, respectively). Under free-choice conditions, testosterone-treated rats failed to complete 0.4±0.3% of trials, while vehicle controls failed to complete 8.4±2.8% ($p<0.05$ vs testosterone). Thus, testosterone-treated rats were not only significantly more likely to select the large/high effort reward lever (Figure 4), they were also more likely to complete a high response requirement to earn the large reward.

**3.2.3 Trial omissions—**In ED, omitted trials were rare in both forced- and free-choice trials throughout the session. Over 5 days of testing with 19 rats, vehicle-treated rats omitted 31 of 4000 trials (<1.0%) and testosterone-treated rats omitted 16 of 3600 trials (<0.5%). There was no effect of effort block on trial omissions ($F_{1,17}=1.39$ $p>0.05$), no effect of drug ($F_{1,17}=0.68$, $p>0.05$), and no drug × effort block interaction ($F_{1,17}=1.05$, $p>0.05$). This result is in contrast to PD, in which rats increased omission of forced-choice trials on the large/uncertain reward lever in response to decreasing probability of reward.

## 4. Discussion

Discounting paradigms evaluate decision making behavior by giving subjects a choice between large and small rewards. All things being equal, rats prefer a large food reward to a smaller one. However, the large reward is “discounted,” or made less desirable, by the imposition of a cost such as a delay or an effort requirement. As the cost associated with the large reward increases throughout the session, rats shift their preference to the smaller non-discounted reward. This behavior is known as a discounting curve. Chronic high-dose testosterone affects decision making by shifting preference for the large discounted reward relative to control subjects. The present study investigated the effects of chronic high-dose testosterone on decision making in response to uncertainty and effort costs. In PD, testosterone decreased risk taking by decreasing preference for the large/uncertain reward compared to vehicle-treated controls. ED showed opposite effects: testosterone-treated rats chose the large/high effort reward significantly more than vehicle controls, indicating decreased sensitivity to physical effort. Likewise, we previously found that testosterone also increased preference for the large discounted reward in delay and punishment discounting (Wood *et al*, 2013; Cooper *et al*, 2014). Taken together, our studies show that testosterone decreases sensitivity to delay, punishment, and effort, while increasing sensitivity to uncertainty. Because testosterone does not always increase preference for the large reward (e.g. PD), testosterone-treated rats are not utilizing a “win-at-all-costs” strategy. Rather, testosterone-treated rats remain sensitive to the different types of costs or risks involved, and testosterone decreases preference for the large discounted reward only in the context of reward uncertainty.

It is unlikely that hunger or satiety can account for testosterone's effects on PD and ED in the present study, or on delay or punishment discounting reported previously (Wood et al, 2013; Cooper et al, 2014). First, as indicated in the Methods, body weights in testosterone- and vehicle-treated rats did not differ throughout the study, and there is no effect of high-dose testosterone on 24-hr food intake in rats fed ad libitum (Wood *et al*, 2013). Secondly, testosterone had opposite effects on PD and ED: testosterone-treated rats were more willing to work for a large reward in ED, but preference for the large reward lever was reduced in

PD. Finally, we know that both vehicle- and testosterone-treated rats do not reach satiety during reward discrimination training when response rates for the large reward lever remain fixed at FR1 across all blocks. In a similar manner, preference for the large reward lever in testosterone-treated rats at high response requirements (FR10, FR15) is not explained by differences in locomotor activity. While activity was not measured in the present study, we have previously shown that high-dose testosterone has no effect on voluntary wheel running (Wood, 2002).

Our hypothesis that testosterone would increase risk taking during PD was not supported. In fact, testosterone-treated rats actually showed increased risk aversion, selecting the large/uncertain reward significantly less than vehicle controls. This was surprising, as testosterone increased selection of the large reward in delay discounting, punishment, and effort discounting. However, PD differs from these other discounting paradigms in an important way. In PD, delivery of the large reward is not guaranteed. In the other three paradigms, the large reward comes with a cost, but the reward itself is certain. Therefore, testosterone may increase tolerance for costs when reward is certain, while decreasing tolerance for reward uncertainty. This possibility is supported by Win-Stay/Lose-Shift analysis (Figure 3). Testosterone-treated rats exhibited a stronger tendency to shift away from the large/uncertain reward lever after incurring a loss, suggesting increased sensitivity to reward omission.

Interestingly, while testosterone significantly decreased preference for the large uncertain reward, it did not have an effect on total pellets earned. Thus, testosterone-treated rats did not perform better or worse than vehicle-treated controls, but simply preferred a strategy that incurred less uncertainty. This is most obvious at the 25% probability block, when total payout from both levers is equal (Figure 2B). Statistically, rats should prefer both levers equally in this probability block, as both levers deliver 8 total pellets. Indeed, vehicle-treated rats choose the large/uncertain reward lever about 50% of the time. However, testosterone-treated rats exhibit a significant aversion to uncertainty compared to controls, choosing the large/uncertain reward on only about 30% of trials in this probability block.

These discounting studies show that testosterone has selective effects on different types of risk taking. Testosterone decreased preference for a large/uncertain reward, but previously increased preference for a large reward paired with footshock (Cooper *et al*, 2014). Therefore, testosterone alters risk taking in a context-dependent manner—increasing tolerance for physical risk (footshock) and decreasing tolerance for risk of reward omission (PD). This result corresponds with studies of human AAS users, which find increased risk taking in the context of physical danger. The types of risks associated with AAS use in humans are physical in nature, such as drinking and driving, carrying a weapon, and not wearing a helmet or seat belt (Middleman *et al*, 1995). Furthermore, increased aggressive behavior seen in testosterone-treated animals in the lab could indicate decreased sensitivity to physical risk (Harrison *et al*, 2000; Wood *et al*, 2013). The decreased risk taking by testosterone-treated rats in PD was surprising, as high endogenous testosterone levels correlate with increased financial risk taking in the stock market and Iowa Gambling task (Coates and Herbert, 2008; Stanton *et al*, 2011). However, the testosterone treatment in this study models AAS use by inducing supraphysiologic levels of circulating testosterone. High

testosterone levels in a normal physiologic range may have different behavioral effects compared to the extremely high levels (up to 100× normal) caused by AAS.

On the other hand, our hypothesis that testosterone would increase preference for the large/high effort reward in ED was supported. While all rats decreased selection of the large reward as the effort requirement increased, testosterone-treated rats exhibited a flatter discounting curve. This means testosterone decreased sensitivity to effort. This conclusion is also supported by the difference in incomplete trials between groups. Not only were testosterone-treated rats more likely to select the large/high effort reward, they were also significantly more likely to complete the response requirement after lever selection. This corresponds with human behavior, as athletes and bodybuilders who abuse AAS must exert extreme physical effort while training to achieve their goals. Compared to previous effort discounting studies (Uban *et al*, 2011; Floresco *et al*, 2008b), vehicle-treated rats in the present study tended to exhibit a stronger preference for the large/high effort reward than vehicle controls in previous studies. This may be due to the large:small reward pellet ratio (see *Methods 2.5.3*), or to sex differences in ED behavior. The female rats in Uban *et al* (2011) were less likely to select the large/high effort reward than the males rats in Floresco *et al* (2008b) and in the present study.

Testosterone may affect behavior on ED and PD via DA in the nucleus accumbens (Acb). A variety of evidence shows that decision making with discounting paradigms depends on DA function in the mesocorticolimbic DA system. For delay, effort, and probability discounting, systemic administration of amphetamine (AMPH) increases selection of the large reward, while DA receptor antagonists decrease large reward selection (Floresco et al, 2008b; St. Onge and Floresco, 2009). With punishment discounting, AMPH has opposite effects: it decreases selection of the large reward paired with footshock (Simon *et al*, 2011). Thus, like testosterone, AMPH has broad, but context-dependent effects on decision making.

The divergent effects of testosterone on PD and ED may depend on different regions of the Acb. Specifically, the core of Acb (AcbC) preferentially modifies ED behavior, while PD is influenced by the shell (AcbSh) (Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011). Inactivation of AcbC decreases selection of the large/high effort reward with ED, but has no effect on PD behavior. In contrast, inactivation of AcbSh decreases selection of the large/uncertain reward in PD, but has no effect on ED behavior (Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011). ED and PD are particularly sensitive to D2 receptor (D2R) function. Systemic D2R antagonism decreases selection of the large reward in both ED and PD (Floresco et al, 2008b; St. Onge and Floresco, 2009). Conversely, systemic stimulation with a D2R agonist increases selection of the large reward in PD (St. Onge and Floresco, 2009). In this regard, 2 weeks of treatment with the long-acting AAS nandrolone decanoate decreases D2R density in AcbSh and increases D2R density in AcbC of young adult rats relative to vehicle-treated control males (Kindlundh *et al*, 2001). Thus, we hypothesize that testosterone may decrease selection of the large reward in PD by decreasing D2R function in the AcbSh, and increase large reward selection in ED by increasing D2R function in AcbC. This could also explain increased risk taking by testosterone-treated rats with punishment discounting (Cooper *et al*, 2014). In punishment discounting, low D2R levels in AcbSh correlate with increased risk taking, and D2R antagonists block the effects

of AMPH on risk taking (Mitchell *et al*, 2014; Simon *et al*, 2011). Therefore, decreased D2R function in AcbSh is consistent with testosterone's effects on both probability and punishment discounting. PD behavior is also dependent on D1 receptor (D1R) function and DA release in Acb. Treatment with D1R antagonists systemically or in Acb and PFC decreases selection of the large/uncertain reward (St. Onge and Floresco, 2009, Stopper et al. 2012, and St. Onge et al, 2011). Likewise, nandrolone decreases D1R density in Acb (Kindlundh *et al*, 2001). Further studies will be necessary to determine the site-specific effects of testosterone on DA and DA receptors in Acb that regulate decision making behavior.

As a mechanism to understand human AAS use, the rat model has some limitations. Human AAS users often engage in intense exercise and physical training. Exercise itself is rewarding (Greenwood et al. 2011), and AAS may increase a user's ability to engage in exercise. While our animal model does not incorporate exercise, it does control for variability in motivation for AAS use. Human users are often motivated by desire to enhance physical appearance or athletic performance. Humans also have variable preexisting behavioral tendencies which may contribute to the initiation of AAS use. Our animal model allows us to make important first steps toward determining AAS effects on decision making by eliminating these confounds. Because all rewards in PD and ED are relatively immediate, these paradigms do not test long-term extinction of behaviors that are no longer rewarding. While we have previously shown that high-dose testosterone impaired cognitive flexibility (Wallin and Wood, 2015), this deficit is unlikely to explain the effects on decision making in the present study. In PD, testosterone-treated rats actually exhibited greater behavioral flexibility, decreasing their preference for the large reward throughout the session significantly more than vehicle-treated controls.

Testosterone's effects on decision making—decreased sensitivity to effort and physical risk, and increased sensitivity to uncertainty—may contribute increased aggression in testosterone-treated rats. Testosterone increases agonistic behavior in a resident-intruder model (Wood *et al*, 2013) where the resident test animal is likely to beat the intruder. Thus, testosterone may increase aggressive behavior by increasing tolerance for physical risk and effort output when reward (winning) is certain. This is consistent with the idea that testosterone-induced aggression (‘roid rage) is not indiscriminate, but remains sensitive to context. For instance, testosterone-treated rats show more aggression in their home-cage than in a neutral arena, and when fighting an intact opponent compared to castrated one (McGinnis *et al*, 2002). Additionally, testosterone does not increase motivation to fight, but does increase aggressive behavior once an intruder male is present (Wood *et al*, 2013). Taken together, these studies suggest that testosterone modulates risk taking and decision making in a context-dependent manner. They further show that adult decision making is impacted by testosterone treatment begun in adolescence. These results will increase understanding of behavioral changes in AAS users and inform the public and health professionals of previously unknown behavioral effects of AAS.

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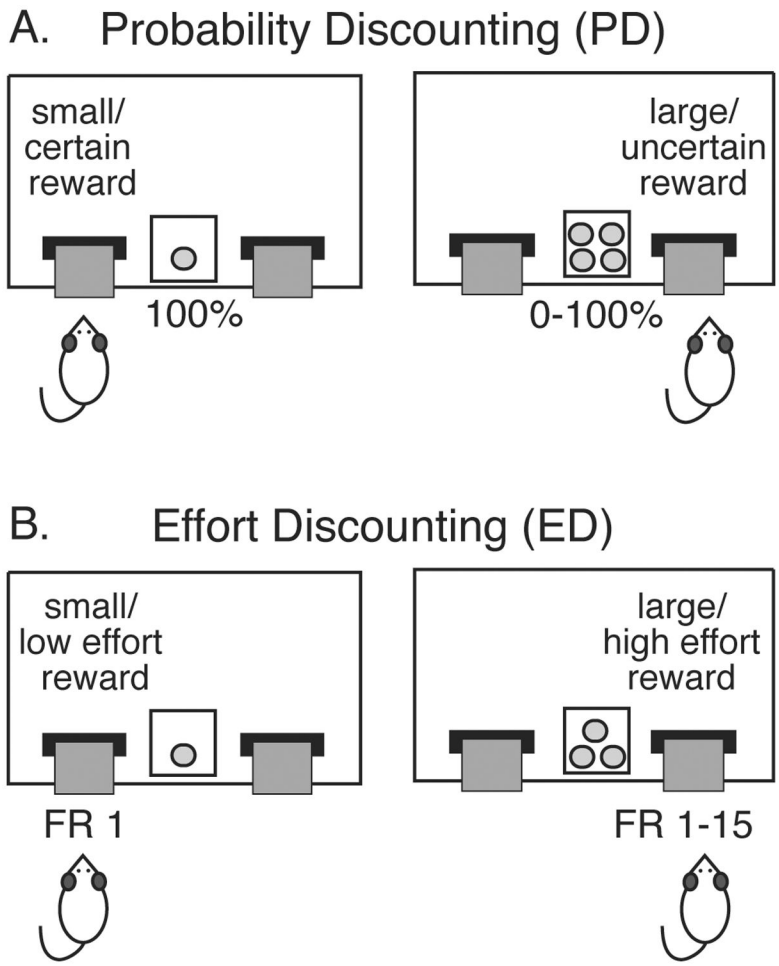


**Figure 1.**

Operant tasks. A) For Probability Discounting (PD), rats choose between a small/certain reward (1 pellet delivered with 100% probability) and a large/uncertain reward (4 pellets delivered with decreasing probability throughout the session). B) For Effort Discounting (ED), rats choose between a small/low effort reward (1 pellet for 1 response) and a large/high effort reward (3 pellets delivered with increasing response requirement throughout the session).

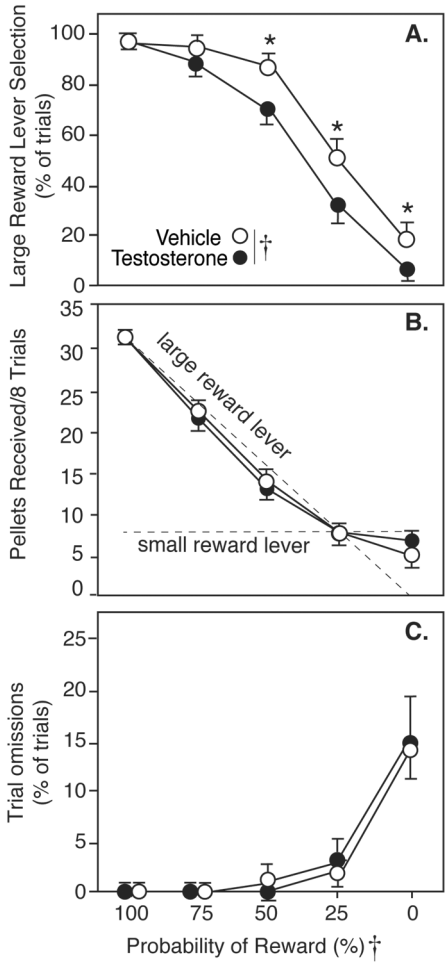


**Figure 2.**

A) Large reward lever selection (mean±SEM) and B) pellets earned by testosterone-(black circles) and vehicle-treated rats (white circles) during 8 free-choice trials in each probability block with Probability Discounting. Dotted lines indicate the total pellets earned by exclusively selecting the large/uncertain reward or small/certain reward lever in each block. C) Trials omitted (mean±SEM) by testosterone- (black circles) and vehicle-treated rats (white circles) during 4 forced-choice trials on the large/uncertain reward lever in each probability block with Probability Discounting. Cross indicates $p<0.05$ by RM-ANOVA. Asterisks indicate $p<0.05$ by Dunnett's test.

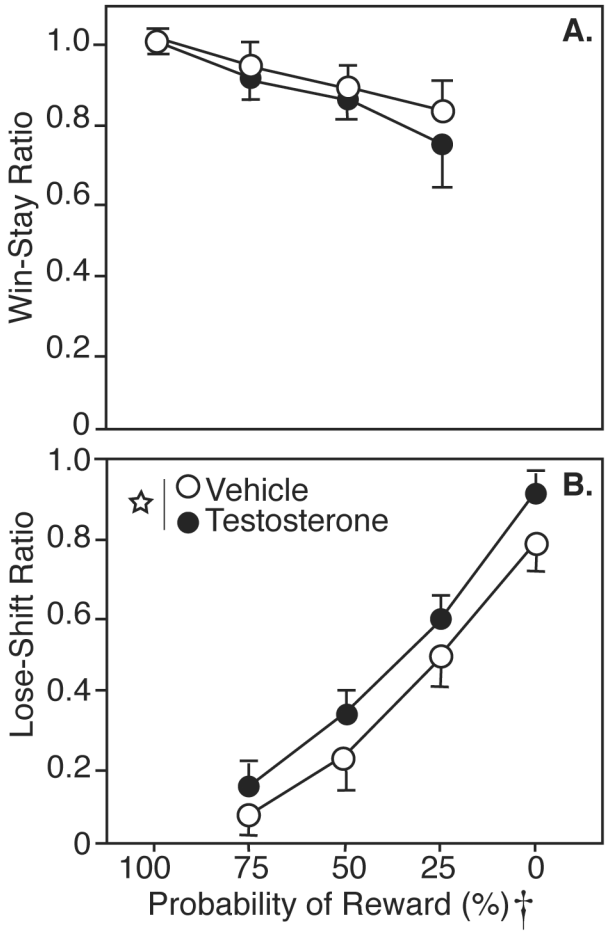


**Figure 3.**

A) Win-Stay and B) Lose-Shift ratios (mean±SEM) for testosterone- (black circles) and vehicle-treated rats (white circles) during 8 free-choice trials in each probability block with Probability Discounting. Cross indicates $p<0.05$ by RM-ANOVA. Star indicates $p=0.07$ by RM-ANOVA

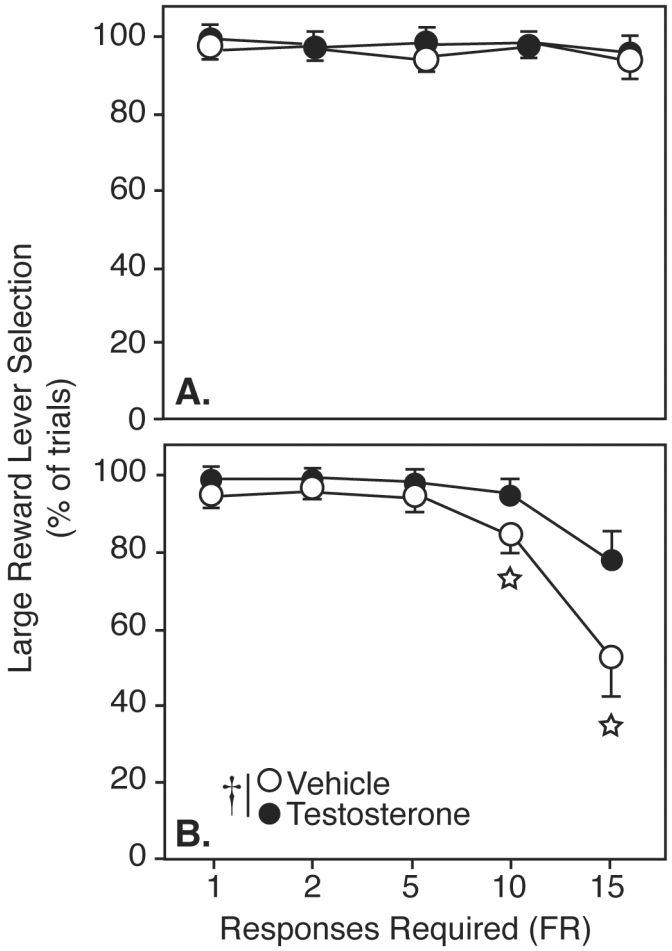


**Figure 4.**

Large reward lever selection (mean±SEM) by testosterone- (black circles) and vehicle-treated rats (white circles) during 8 free-choice trials in each block of A) Reward Discrimination and B) Effort Discounting. Crosses indicate $p<0.05$ by RM-ANOVA. Stars indicate $p=0.07$ by Dunnett's test.

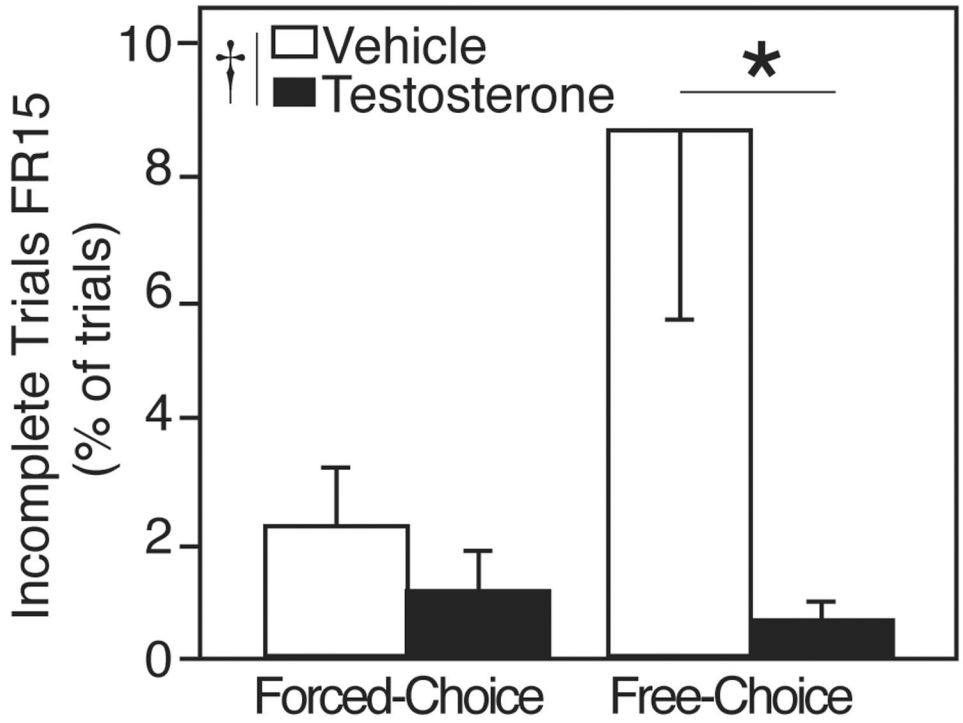


**Figure 5.**

Incomplete trials (mean±SEM) by testosterone- (black bars) and vehicle-treated rats (white bars) during forced-and free-choice trials in the last effort block (FR15) of Effort Discounting. Cross indicates $p<0.05$ by RM-ANOVA. Asterisk indicates $p<0.05$ by Student's t-test.