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ANABOLIC-ANDROGENIC STEROIDS AND COGNITIVE EFFORT DISCOUNTING IN MALE RATS.

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Abstract

Anabolic-androgenic steroids (AAS) are drugs of abuse that impair behavior and cognition. In a rodent model of AAS abuse, testosterone-treated male rats expend more physical effort, by repeatedly pressing a lever for a large reward in an operant discounting task. However, since modern society prioritizes cognitive over physical effort, it is important to determine if AAS limit cognitive effort. Here we tested the effects of AAS on a novel cognitive-effort discounting task. Each operant chamber had 3 nose-pokes, opposite 2 levers and a pellet dispenser. Rats pressed a lever to illuminate 1 nose-poke; they responded in the illuminated nose-poke to receive sugar pellets. For the 'easy' lever, the light remained on for 1s, and a correct response earned 1 pellet. For the 'hard' lever, the light duration decreased from 1s to 0.1s across 5 blocks of trials, and a correct response earned 4 pellets. As the duration of the nose-poke light decreased, all rats decreased their choice of the hard lever in a modest discounting curve. Task accuracy also decreased significantly across the 5 blocks of trials. However, there was no effect of testosterone on choice of the hard lever or task accuracy. Antagonism of dopamine D1 or D2 receptors had no effect on lever choice or task accuracy. However, serotonin depletion significantly decreased preference for the hard lever, and impaired task accuracy. Thus, physical effort discounting depends on dopamine activity, while cognitive effort discounting task is sensitive to serotonin. AAS impair physical effort discounting, but not cognitive effort discounting.

Keywords

anabolic agents; decision making; dopamine; operant behavior; testosterone; serotonin

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1. Introduction

Anabolic androgenic steroids (AAS) are performance-enhancing substances taken to increase muscle mass and enhance athletic performance. Although AAS have legitimate medical applications, illicit use is a growing concern with over 4 million users in the United States alone (Kanayama and Pope, 2018). Importantly, AAS use is no longer restricted to professional athletes, but now includes both adolescents and young adult men seeking cosmetic benefits (Kanayama and Pope, 2018; Pope et al., 2014). In this regard, 22% of AAS users initiate use before age 20, and 85% before age 35 (Pope et al., 2014). AAS abuse has serious consequences for physiological health, including increased risk for reproductive, cardiovascular, hepatic, and psychiatric dysfunction (Kanayama et al., 2018). Deficits in visuospatial memory tasks have been observed in long-term human AAS users (Kanayama et al., 2013), and early exposure to AAS during adolescence is especially troubling, since neural circuitry is still undergoing maturation, and AAS exposure may have a lifelong impact (Blakemore and Choudhury, 2006; Cunningham et al., 2013; Cunningham and McGinnis, 2007). Our laboratory focuses on AAS-induced changes in cognition and decision-making in rodents. Early exposure to AAS in adolescent male rats causes long-term changes in behavior and aggression (Lumia and McGinnis, 2010). However, the full extent of AAS use on cognitive function remains unknown. This was the focus of the present study.

Studying the effects of AAS on cognition in human users is challenging, given the diversity of AAS types, and the unique pattern of dosing and timing by individual users. For this reason, we model AAS abuse in male rats exposed to chronic high-dose testosterone to standardize the type, dose, and timing of exposure. Testosterone exposure begins in adolescence at 5 weeks of age (Spear, 2000), and extends through young adulthood to model human patterns of AAS use (Pope et al, 2014). Testosterone treatment may have different effects on decision making if not initiated until adulthood. We use testosterone because it is a popular AAS used recreationally due to its low cost and easy availability, and because all AAS are synthetic derivatives of testosterone. In our recent studies, chronic testosterone treatment beginning in adolescence impairs cognitive flexibility (Wallin and Wood, 2015) and modifies decision-making in operant discounting tasks in rats (Cooper et al., 2014; Wallin et al., 2015; Wallin-Miller et al., 2018; Wood et al., 2013). In operant discounting, an animal chooses between two levers, one associated with a small reward (1 sugar pellet), and the other with a large reward (3 or 4 pellets). The large reward is discounted by the imposition of a “cost” to make the reward less desirable. As the cost associated with choice of the large reward increases across blocks of trials, preference for the lever associated with the large reward decreases, to create a discounting curve. Compared with vehicle controls, testosterone-treated rats continue to show greater preference for the large reward lever when the discounting cost is a delay (Wood et al., 2013), mild punishment (Cooper et al., 2014), or physical effort (Wallin et al., 2015). However, testosterone-treated rats are more sensitive to reward uncertainty (Wallin et al., 2015). Therefore, testosterone-treated rats do not favor a “win-at-all-costs” strategy. To extend these findings, the present study explored the effects of chronic, high-dose testosterone on the willingness to expend cognitive effort. Studying the effects of AAS on cognitive effort is important because success in modern society emphasizes cognitive ability over physical activity.

Cocker et al. (2012) developed a rodent cognitive effort task (rCET), where a rat chooses to respond on a lever associated with an easy or difficult task to earn a small or large reward, respectively. After selecting a lever, the rat had to respond in the correct nose-poke, which was illuminated for either 1.0s (easy trial) or 0.2s (hard trial). We adapted this approach to create a cognitive effort-discounting task, where the cost associated with the large reward (attention to a progressively shorter light duration) increases in successive blocks of trials. This task measures two features of cognitive effort: 1) the rat's willingness to expend cognitive effort, as determined by their choice of the hard lever associated with a large reward, and 2) their accuracy in completing the task. Since testosterone-treated rats will expend more physical effort for a large reward (Wallin et al., 2015), we hypothesized that they would also expend more cognitive effort.

Many aspects of decision-making behavior depend on the activity of dopamine (DA) D1-like (D1R) and D2-like (D2R) receptors in the nucleus accumbens (Acb; Ghods-Sharifi and Floresco, 2010; Hosking et al., 2015; Stopper and Floresco, 2011; Stopper et al., 2013; Winstanley and Floresco, 2016). Specifically, antagonism of D1R in Acb increases sensitivity to reward omission in a test of probability discounting (Stopper et al, 2013), and both D1R and D2R antagonists in Acb reduce operant responding for food in a cost-benefit test (Nowend et al, 2001). Likewise, AAS increase sensitivity to reward omission and alter effort-based decision making (Wallin et al., 2015). In parallel with these behavioral effects, AAS decrease D1R throughout Acb, and selectively reduce D2R in the shell of Acb (Kindlundh et al. 2001). Accordingly, the present study tested dopaminergic involvement in cognitive effort discounting in testosterone- and vehicle-treated rats. On the other hand, D1R and D2R antagonists have no effect on performance in the rCET (Hosking et al, 2015). However, serotonin also plays a role in decision-making. Systemic serotonin depletion increases impulsivity measured by reduced preference for a large delayed reward in rats (Denk et al., 2005), and promotes risky decision making in rats (Zeeb et al., 2009) and monkeys playing a gambling task (Long et al., 2009). Thus, we also tested serotonergic involvement in cognitive effort discounting using systemic serotonin depletion.

2. Materials and methods

2.1. Animals

28 male Long-Evans rats (Charles River Laboratories, MA) were 5 weeks of age at the start of the study. Rats were pair-housed with access to water *ad libitum* under a reversed 14L: 10D photoperiod. Rats were tested daily (5 d/week) during the dark phase, and all rats remained gonad-intact in order to approximate human AAS use. To maintain a slow rate of growth (3–4 g/day) and facilitate operant responding, rats were food restricted as in our previous studies (Cooper et al., 2014; Wallin et al., 2015). Rats were weighed daily and received ~15–25 g of chow in their home cage following behavioral testing. Vehicle- and testosterone-treated groups did not differ in body weight at the start of the study or throughout testing. With free-feeding, there is no effect of chronic testosterone treatment on food intake or body weight (Wood et al, 2013). 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, 8th Ed (National Research Council, National Academies Press, Washington DC; 2011).

2.2. Testosterone treatment

Starting 2 weeks before behavioral training and throughout testing, rats received injections s.c. of testosterone (7.5 mg/kg; 4-androsten-17β-ol-3-one; Steraloids, RI) or aqueous vehicle [3% ethanol and 13% cyclodextrin (RBI, MA)] 5 d/week. This dose approximates heavy steroid use in humans and has been used previously to demonstrate the effects of AAS on cognition and decision-making in rats (Cooper et al., 2014; Wallin et al., 2015; Wallin and Wood, 2015; Wood et al., 2013). At 7.5 mg/kg, testosterone cypionate causes a ten-fold increase in serum testosterone (>20 ng/ml), vs oil-treated control rats (ca. 2 ng/ml; Clark et al, 1997). However, it is important to note that chronic exposure to AAS lowers endogenous testosterone secretion. Injections were administered immediately prior to behavioral training and testing.

2.3. Operant chambers

All training and testing was conducted in operant chambers (Med Associates, VT) enclosed in sound-attenuating boxes with fans for ventilation. As shown in Figure 1A, one side of each chamber was outfitted with three nose-pokes with stimulus lights and infrared beams. The opposite wall had 2 retractable levers flanking a pellet dispenser with food cup and house light.

2.4. Training

Initially, rats were trained in daily 40-min sessions to respond in an illuminated nose-poke to receive a 45-mg sucrose pellet (Bio-Serv Inc., Frenchtown, NJ). In each trial, one nose-poke light was illuminated and remained lit until the rat made a response in any of the three nose-pokes. A correct response in the illuminated nose-poke earned a sucrose pellet. An incorrect response extinguished the nose-poke light, and the chamber returned to the inter-trial interval (ITI) state with the house light on for 5s before the start of a new trial. Once rats demonstrated 90% correct responses, they were required to respond in the illuminated nose-poke within 30s; response omissions resulted in a 5s ITI. Subsequently, the nose-poke light duration was reduced to 20s, and then to 10s.

After rats responded in the correct nose-poke within 10s with 90% accuracy, they were trained to respond on a lever (Figure 1A). One retractable lever was inserted into the chamber at random and remained extended until the rat responded. Following a lever response, the lever retracted, the house-light was extinguished, and one of the nose-pokes was randomly illuminated after a 5s delay. Rats had 10s to respond in the illuminated nose-poke to receive a pellet. When rats completed at least 60 trials in 40 min with 90% accuracy, the nose-poke light duration was decreased to 5s, then to 2s, and 1s. Although the nose-poke light was only illuminated briefly, rats had 5s to make a response before the trial was recorded as an omission.

Lastly, rats were next trained on reward discrimination, consisting of 60 trials divided into 5 blocks. Each block included 4 forced-choice trials and 8 free-choice trials. In forced-choice

trials, 1 lever was inserted (2 trials/lever). In free-choice trials, both levers were available. One lever was defined as the 'easy' lever and the other as the 'hard' lever. The position for each remained the same throughout training and testing. However, no cognitive effort cost was imposed for a response on the hard lever during reward discrimination, and the nose-poke light was illuminated for 1s with a response on either lever across the five blocks. A correct response on the easy lever delivered 1 pellet; a correct response on the hard lever delivered 3 pellets. Training was complete when rats responded on the hard lever on 90% of free-choice trials.

2.5. Cognitive effort discounting

Once rats mastered reward discrimination, they were tested for cognitive effort discounting for 10 days until choice behavior stabilized. As in reward discrimination, each daily session consisted of 60 trials divided into 5 blocks of 4 forced-choice and 8 free-choice trials. Likewise, correct response in the illuminated nose-poke following choice of the easy lever delivered 1 pellet; a correct response following choice of the hard lever delivered 3 pellets. However, the hard lever was now discounted by decreasing the time the nose-poke light remained illuminated across blocks: 1.0, 0.6, 0.4, 0.2, and 0.1s (Figure 1B). Rats had 90 minutes to complete 60 trials. Responses were considered stable when there was no effect of time by repeated-measures ANOVA (RM-ANOVA) comparing choice of the hard lever at each block over 3 days of testing, with time as the repeated measure, as in Cooper et al. (2014).

2.6. Monoamine drugs

2.6.1. D1 and D2 receptor antagonists—

On the day after baseline choice behavior was established, rats were injected with saline, and tested for cognitive effort discounting 10 minutes later. Subsequently, all rats were tested with D1 and D2 receptor antagonists at two doses in a counter-balanced design on separate test days with a baseline saline test day between injections. To control for potential additive effects, none of the rats received both doses of the same antagonist on consecutive days. Saline baseline behavior was evaluated on separate days from monoamine drug manipulations. DA antagonists were the D1R antagonist R(+)-SCH-23390 hydrochloride (SCH; #D054 Sigma-Aldrich, St. Louis, MO) and the D2R antagonist S-(-)-eticlopride hydrochloride (eticlopride; #E101; Sigma-Aldrich). SCH and eticlopride solutions were prepared fresh daily in 0.9% physiological saline, and were delivered by injection i.p. 10 minutes prior to behavior. SCH was used at 0.01 mg/kg and 0.03 mg/kg, and eticlopride at 0.03 mg/kg and 0.06 mg/kg. These doses have previously been shown to affect cognition in rats (Floresco et al., 2006; Hosking et al., 2015; St Onge and Floresco, 2009).

2.6.2. Serotonin Depletion—

After exposure to DA receptor antagonists, rats underwent serotonin depletion with 4-chloro-DL-phenylalanine (PCPA; 300 mg/kg in saline; #C6506, Sigma-Aldrich). PCPA acts as an irreversible inhibitor of serotonin synthesis. Rats received PCPA injections i.p. at 48 and 24 hours before testing. This treatment is sufficient to deplete 90% of serotonin in the central nervous system (Brigman et al., 2010; Izquierdo et al., 2012). Rats continued to receive daily injections of testosterone or cyclodextrin vehicle immediately before training and testing throughout the study.

2.7. Data analysis

Data were collected using MedPC software and imported into Microsoft Excel. Statistical analysis was conducted using SPSS version 20 statistical software. Statistical significance was set to $p<0.05$. Saline baseline data were computed from the average of 4 days of testing (pre-treatment plus 3 rest days). Data on total trials completed, total pellets earned, and total omissions were evaluated by ANOVA comparing vehicle and testosterone at saline baseline. Because lever(s) were extended indefinitely in each trial until the rat made a response, the trial duration was not fixed. Therefore, the total number of forced-choice and free-choice trials completed in 90 minutes reflects attention to the operant task. For the monoamine drugs (SCH, eticlopride, PCPA), RM-ANOVA with drug as the repeated measure (saline vs. drug) was used to test for both drug and testosterone effects on total trials, total pellets, and total omissions. Each monoamine drug was independently compared to saline. If a significant effect was found, estimate of effect size was calculated and reported as η^2, using the appropriate test as specified in Lakens (2013).

Percent choice of the hard lever during choice trials was calculated from the total number of trials in which the hard lever was selected out of 8 choice trials in each block. Percent accuracy on the hard lever was calculated from the number of successful trials, out of the total number of choice trials in which the hard lever was selected in each block. For saline baseline, choice behavior and task accuracy were analyzed by RM-ANOVA with testosterone (vehicle vs. testosterone) as the between-subjects factor, and block as the repeated measure. For the monoamine drugs, choice behavior and task accuracy were analyzed with a three-factor mixed RM-ANOVA with testosterone (vehicle vs. testosterone) as the between-subjects factor, and block and drug (saline vs. monoamine drug) as repeated measures.

At baseline, all rats completed 60 trials in 90 minutes. During treatment with monoamine drugs, some rats failed to complete all 60 trials. For rats that did not complete any choice trials in a particular block, no data were recorded for that block, and this reduced the number of subjects analyzed by RM-ANOVA (see Results).

To determine whether testosterone or monoamine drugs affected sensitivity to reward delivery and omission, win-stay (WS) and lose-shift (LS) behavior was analyzed on a trial-by-trial basis. A WS occurred when the rat chose the hard lever and received the large reward for a correct response (win), and then chose the hard lever again in the following trial (stay). LS occurred when the rat received no pellets on a hard lever trial (loss), either because of an incorrect response or omission, and then chose the easy lever 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 block and compared by three-factor mixed RM-ANOVA with testosterone treatment as the between-subjects factor and block and drug (saline vs. monoamine drug) as the repeated measures.

3. Results

3.1. Effect of testosterone at baseline

There was no effect of testosterone on task acquisition. Both vehicle- and testosterone-treated rats required the same number of training sessions to learn the task and completed all 60 trials within the 90-minute session. Testosterone- and vehicle-treated rats earned a comparable number of pellets (vehicle: 74.9±6.2 pellets/session vs. testosterone: 82.1±4.4) and made a similar number of omissions (vehicle: 19.3±2.4 vs. testosterone: 16.5±1.9) in each session.

For all rats, there was a significant effect of block on choice of the hard lever [$F_{(4,14)}$=6.99, p<0.003; η^2=0.39], but no effect of testosterone and no testosterone x block interaction by RM-ANOVA. All rats (vehicle- and testosterone-treated) responded less frequently on the hard lever as the nose-poke light duration decreased (Figure 2A). Rats chose the hard lever on 89.5±1.8% of free-choice trials at a 1s light duration, but only on 71.9±4.0% of trials with a 0.1s light duration. Likewise, there was a significant effect of block on task accuracy [$F_{(4,14)}$=29.43, p<0.0001; η^2=0.89], but no effect of testosterone (Figure 2B). Vehicle- and testosterone-treated rats showed a marked impairment in accuracy on hard lever trials as the nose-poke light duration decreased, from 74.9±2.5% correct when the duration was 1s to only 28.6±4.3% correct at 0.1s light duration.

As illustrated in Figure 2C, the WS ratio remained unchanged throughout all five blocks of trials, and there was no effect of testosterone treatment. Although task accuracy declined steeply as the duration of the nose-poke light decreased, rats retained a strong preference for the hard lever following a win, with a WS ratio of 0.92±0.02 when the nose-poke light duration was 1s, and 0.94±0.04 when the duration was 0.1s. Similarly, rats were disinclined to shift to the easy lever after a loss on the hard lever, demonstrating a low LS ratio in the first 1.0s block (0.11±0.05). Nonetheless, the LS ratio increased significantly across blocks [$F_{(4,13)}$=4.03, p<0.05; η^2=0.55], to 0.35±0.05 when the nose-poke light duration was 0.1s (Figure 2D).

3.2. D1R antagonist, SCH

At 0.01 mg/kg, there was no effect of the D1R antagonist SCH on any measure of lever choice or task accuracy, and no interaction with testosterone (data not shown). At 0.03 mg/kg, SCH significantly reduced the number of trials completed, to 51.5±1.7 trials in 90 min [$F_{(1,17)}$=6.29 p<0.05; η^2=0.27], but there was no effect on total pellets received (71.4±7.0 per session) or total omissions (16.0±2.4).

For analysis by RM-ANOVA, only 12 rats (n=6 each, vehicle and testosterone) had data from all 5 blocks of choice trials; but restricting analysis to the first 4 blocks increased the sample size to 15 rats (7 vehicle, 8 testosterone). We therefore analyzed the data twice: with n=12 from all 5 blocks, and again with n=15 including just the first 4 blocks of choice trials. The results were identical. There was no effect of 0.03 mg/kg SCH on hard lever choice, and no interaction with testosterone. Both vehicle- and testosterone-treated rats maintained a strong preference for the hard lever (Figure 3A). However, after SCH treatment there was no longer an effect of block on hard lever choice. D1R antagonism had no effect on task

accuracy, which decreased significantly for all rats across trial blocks [$F_{(4,7)}$=13.80, p<0.002; η^2=0.89 for 5 blocks] (Figure 3B). Furthermore, there was no interaction of SCH with testosterone. WS and LS ratios were unaffected (data not shown).

3.3. D2R antagonist, Eticlopride

At 0.03 mg/kg, the D2R antagonist eticlopride had no significant effect on the number of trials completed, pellets earned or number of omissions. Likewise, there was no effect of eticlopride and no interaction with testosterone on hard lever choice or task accuracy. At 0.06 mg/kg, eticlopride decreased the number of choice trials completed (40.7±4.7) [$F_{(1,17)}$= 15.95, p<0.001; η^2=0.43] and pellets earned (50.5±8.3) [$F_{(1,17)}$=12.70, p<0.005; η^2=0.35] for all rats. Although there was a modest decrease in omissions (14.3±2.0 vs. 17.8±1.5 for saline), this did not reach significance [$F_{(1,17)}$ =3.85, p=0.066; η^2=0.18

Similar to SCH, there was no effect of 0.06 mg/kg eticlopride on hard lever choice across 5 (n=9) or 4 blocks of choice trials (n=10), and no interaction with testosterone. Following D2R antagonism there was no longer a significant effect of block on hard lever choice (Figure 3C). Despite this effect, task accuracy decreased as the nose-poke light duration decreased [$F_{(3,6)}$=8.32, p<0.015; η^2=0.81 for 4 blocks; $F_{(4,4)}$=6.00, p=0.055; η^2=0.86 for 5 blocks] (Figure 3D), but there was no effect of eticlopride on task accuracy in vehicle- or testosterone-treated rats. In addition, eticlopride had no significant effect on either WS or LS ratios (data not shown).

3.4. Serotonin Depletion with PCPA

Relative to saline baseline, rats treated with PCPA completed significantly fewer trials (51.0±2.7) [$F_{(1,17)}$=8.45, p < 0.01; η^2=0.33], earned fewer pellets (78.7±3.7 saline vs. 41.3±3.9 PCPA) [$F_{(1,17)}$=166.12, p<0.0001; η^2=0.91], and had more response omissions (17.8±1.5 saline vs. 27.6±2.4 PCPA) [$F_{(1,17)}$=15.585, p<0.001; η^2=0.48] in each 90-min session. Even so, there was no significant interaction of testosterone with PCPA.

Across all 5 blocks of trials (n=10), PCPA treatment marginally decreased choice of the hard lever [$F_{(1,8)}$=4.81, p=0.06; η^2=0.38] as shown in Figure 4A, although this did not reach significance. Furthermore, there was no effect of block on choice of the hard lever. When analysis was restricted to the first 4 blocks (n=14), PCPA significantly decreased choice of the hard lever [$F_{(1,12)}$=9.88, p<0.01; η^2=0.45]. As shown in Figure 4B, serotonin depletion significantly reduced task accuracy [$F_{(1,8)}$=115.05, p<0.0001; η^2=0.93 for 5 blocks], and eliminated the effect of block. Overall, serotonin depletion significantly decreased choice of the hard lever and impaired accuracy. However, there was still no effect of testosterone-treatment on either measure. We were unable to compute WS or LS ratios because of incomplete data.

4. Discussion

The present study used a novel cognitive effort discounting task to determine if chronic exposure to high-dose testosterone affects the willingness or ability to expend cognitive effort to obtain a food reward. As the duration of the nose-poke light decreased, response accuracy declined, along with preference for the hard lever to obtain a large reward.

However, testosterone had no effect on lever preference or accuracy. The lack of an effect of testosterone on cognitive effort discounting contrasts with our recent finding (Wallin et al., 2015) that testosterone-treated rats are willing to expend more physical effort (i.e. make more lever presses) to obtain a large reward. Furthermore, although physical effort discounting is sensitive to DA manipulations via both D1R and D2R (Salamone et al., 2009; Salamone et al., 2012), cognitive effort discounting in the present study was unaffected by the DA antagonists SCH and eticlopride. Instead, serotonin depletion with PCPA reduced preference for the hard lever and impaired performance on hard lever trials. These findings highlight the selective effects of testosterone on DA-dependent decision-making and emphasize that cognitive and physical effort discounting may be mediated by different neurochemical systems.

Our cognitive effort discounting task is modified from the rodent cognitive effort task (rCET) of Cocker et al. (2012). In both tasks, the rat chooses between two levers to select trial difficulty, i.e. the nose-poke light duration. In rCET, the duration in a high-effort trial is fixed at 0.2s. By contrast in the present study, the duration decreases from 1.0–0.1s across successive blocks. In their study, Cocker et al (2012) divided their subjects into ‘workers’ who preferred the high-effort/high-reward lever, and ‘slackers’ who preferred the low-effort/low-reward lever.

Interestingly, we did not find separate populations of ‘workers’ and ‘slackers’ in the present study; all rats preferred the hard lever. This could be due either to the greater visuospatial cognitive demands of rCET (choice of 5 nose-pokes vs. 3 in cognitive effort discounting) and/or the larger reward in our task (3 pellets vs. 2 pellets in rCET). Thus, compared with rCET, cognitive effort discounting in the present study favors a response on the hard lever. Even so, there was still a significant effect of block on both hard lever choice and accuracy at baseline. This suggests that testosterone- and vehicle-treated rats adjust their responses similarly in accordance with the likelihood of success. The lack of effect of testosterone or testosterone x block interaction argues that chronic high-dose testosterone does not influence choice behavior or performance on cognitive effort discounting.

Cognitive effort discounting extends our understanding of the impact of AAS on decision-making in a rat model of AAS abuse. From our previous studies, testosterone-treated rats will work harder (physical effort discounting; Wallin et al., 2015), wait longer (delay discounting; Wood et al., 2013), and accept more discomfort (punishment discounting; Cooper et al., 2014) to earn a large reward, compared with vehicle-treated controls. Rats treated chronically with high-dose testosterone also show impairments in cognitive flexibility, as measured by set-shifting and reversal learning (Wallin and Wood, 2015). However, testosterone-treated rats are relatively less tolerant of uncertainty (probability discounting; Wallin et al., 2015), their preference for a large uncertain reward is lower than that of controls.

Cognitive effort discounting is intriguing because it shares elements in common with both physical effort and probability discounting. Similar to physical effort discounting, cognitive effort discounting requires the rat to complete a task after selecting the large reward lever in order to earn a reward. In physical effort discounting, testosterone-treated rats selected the

large reward lever more often and completed more trials, compared with vehicle controls (Wallin et al., 2015). In contrast, testosterone- and vehicle-treated rats in the present study showed equivalent preference for the hard lever, with similar numbers of omissions. These findings suggest that cognitive effort and physical effort may be mediated by different neurochemical circuits, as discussed below. Secondly, like probability discounting, choice of the hard lever in cognitive effort discounting does not guarantee reward delivery. In probability discounting, rats learn to 'play the odds' by estimating the probability of reward. They then use this information to select the appropriate lever. For cognitive effort discounting, receipt of the large reward depends on the rat's ability to successfully complete the task, where lever choice is informed by the rat's estimate of his own 'skill'. Since AAS users describe feelings of invincibility (van Amsterdam et al., 2010; Vassallo and Olrich, 2010), we expected that chronic high-dose testosterone might cause rats to overestimate their skill in cognitive effort discounting, and thereby choose the hard lever even when it was no longer advantageous to do so. This was not the case, and there was no difference between vehicle- and testosterone-treated rats. Instead our findings add to a growing literature to suggest that AAS-induced changes in behavior (e.g. AAS-induced aggression, colloquially known as 'roid rage) are not irrational and impulsive, but are rather an outcome produced by a different weighing of costs and benefits.

WS and LS ratios for cognitive effort discounting offer additional insight into how rats perceive their skill on the visuospatial task to inform their choice. There was no effect of block on the WS ratio in the present study. Rats continued to prefer the hard lever (72% of trials at 0.1s duration), especially following a win on the previous trial (94%), even when the likelihood of success was low (29%). By contrast, the WS ratio decreased across blocks in probability discounting (Wallin et al., 2015). Specifically, with a comparable chance of success (25%), rats chose the large reward lever on only 51% of trials, including 85% of trials following a win. Differences between the two discounting tasks may account for the differences in WS ratios. After choosing a lever, cognitive effort discounting requires an active response by the rat to earn the large reward, whereas receipt of the large reward in probability discounting is passive. Therefore, choosing the large reward lever in cognitive effort discounting presumably reflects the rat's estimation of his ability to be successful in the task.

The LS ratio for both probability discounting and cognitive effort discounting increased significantly across blocks, indicating that rats are sensitive to reward omission in both tasks. However, the LS ratio in the present study remained relatively low. Specifically, at a 0.1s nose-poke light duration, the LS ratio was 35% vs. 50% for probability discounting when the probability of reward was 25% (Wallin et al., 2015). Together, the higher WS ratio and lower LS ratios with cognitive effort discounting suggest that rats may overestimate their own abilities when the outcome depends on skill (cognitive effort discounting) compared to chance (probability discounting). This has parallels with the human tendency to overestimate their skill in games (Kwak, 2016), and value rewards more when they have to work hard to obtain them (Zentall, 2015).

The lack of an effect of testosterone on cognitive effort discounting was somewhat surprising, considering that AAS alter DA receptors in the nucleus accumbens (Acb), and

that Acb is critical for decision-making (Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011). The Acb core subregion is especially important for rCET and physical effort discounting (Hosking et al, 2015). On the rCET, inactivation of Acb core with baclofen/muscimol severely impaired rats' performance by significantly reducing the number of trials initiated, and tended to decrease choice of the high effort/high reward lever (Silveira et al., 2018). For physical effort discounting, inactivation of Acb core reduced selection of the high effort lever (Ghods-Sharifi and Floresco, 2010). Likewise, stimulating DA activity systemically with amphetamine decreased physical effort expenditure, as did systemic treatment with either the DA receptor antagonist flupenthixol (Floresco et al., 2008), SCH or eticlopride (Hosking et al, 2015). AAS increase D2 receptors in Acb core (Kindlundh et al., 2001) and increase preference for the large reward lever in physical effort discounting (Wallin et al., 2015). In contrast with physical effort discounting, performance on rCET was not affected by systemic administration of SCH or eticlopride (Hosking et al., 2015). This is in keeping with the present study, where there was no effect of systemic treatment with either D1R or D2R antagonists. We cannot rule out the potential for local effects of DA receptor antagonists in Acb core on cognitive effort discounting. Nonetheless, our results and those of Hosking et al (2015) suggest that cognitive effort-based decision making is relatively less sensitive than physical effort discounting to systemic DA manipulations.

Although SCH and eticlopride did not alter preference or accuracy with the hard lever, they did significantly reduce the number of trials completed in 90 minutes. This is in line with the results of Hosking et al. (2015) for rCET, where SCH and eticlopride reduced the number of completed trials, and increased response and choice omissions. In both the present study and rCET, response omissions occur when the rat fails to respond in a nose-poke within 5s after selecting an easy or hard trial. In rCET, choice omissions occur when the rat fails to respond on either lever within 10s. In rCET, rats were tested for 30 min in each session. By contrast, choice omissions did not occur in the present study, because there was no time limit on the lever response. This simplified the analysis of responses across multiple blocks. However, delays in choosing a lever prevented some rats from completing all 60 trials in a 90-min session. 90 min was a generous time allowance, considering that all 60 trials could be completed in less than 18 min with optimal performance. Because some rats treated with SCH or eticlopride did finish all 60 trials, it is unlikely that motor ability was substantially impaired. Instead, it seems more likely that the DA antagonists reduced engagement in the task.

While DA had no effect on cognitive effort discounting, serotonin depletion with PCPA significantly decreased the number of trials completed, decreased preference for the hard lever, impaired task accuracy, and increased response omissions. The 300 mg/kg dose was selected based on previous studies that found no effect on reversal learning (Brigman et al., 2010), or performance in a physical effort task (Denk et al., 2005). Furthermore, the decrease in choice of the hard lever cannot simply be explained by a decrease in preference for a large reward. Previous studies have shown that PCPA-treated rats continue to prefer a large reward in a physical effort T-maze task, but not in a delay task unless the delay costs were matched for both the high and low reward (Denk et al., 2005; Izquierdo et al., 2012). This suggests that serotonin depletion does not affect motivation to expend physical effort

but does contribute to reduced tolerance for delay (Denk et al., 2005). The implication for cognitive effort discounting in the present study is that the decrease in choice of the hard lever following PCPA-treatment may be due primarily to impaired task performance. Previous work has found that serotonin depletion impaired performance in both novel object recognition and spontaneous alternation tasks (du Jardin et al., 2014; Pehrson et al., 2012). More so, short-term and spatial-working memory in rats is impaired by lesions of serotonergic neurons with the serotonin specific neurotoxin 5,7-DHT (Hritcu et al., 2007). Deficits in short-term and spatial-working memory as a result of serotonin depletion could explain the marked decrease in task accuracy in cognitive effort discounting, with subsequent reduced choice of the hard lever.

Nonetheless, PCPA did not reveal effects of testosterone on cognitive effort discounting. AAS abuse has serious psychological and behavioral effects, including changes in mood and aggression (Cunningham and McGinnis, 2007; McGinnis, 2004). The neuromodulator serotonin and its various receptor subtypes may play a role in mediating many of these behavioral effects of AAS (Keleta et al., 2007; Kindlundh et al., 2003; McGinnis, 2004). Animal models of AAS abuse have found changes in receptor densities for the 5-HT1b and 5HT2 receptor (Kindlundh et al., 2003), and lower levels of serotonin in the basal forebrain and dorsal striatum (Lindqvist et al., 2002). AAS have also been shown to downregulate serotonin receptor messenger RNA in the prefrontal cortex and amygdala (Ambar and Chiavegatto, 2009). The relationships among serotonin levels, specific receptors, AAS and aggression have been well studied (Bonson et al., 1994; Bonson and Winter, 1992), with lower levels of serotonin correlated with increased aggression (Grimes and Melloni, 2005; Keleta et al., 2007; Morrison et al., 2015a, b; Ricci et al., 2012; Ricci et al., 2006). Considering the impact of AAS on serotonin receptor expression, it was reasonable to expect that serotonin depletion might reveal effects of testosterone on cognitive effort discounting. Because testosterone- and vehicle-treated rats responded similarly to PCPA, our results instead highlight the overall importance of serotonin availability for cognitive effort discounting. Ultimately, although chronic high-dose testosterone leads to changes in DA and serotonin systems in the brain, these changes do not affect performance on cognitive effort discounting. Instead, cognitive effort discounting as presented here is unaffected by DA antagonism, but sensitive to serotonin depletion.

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Highlights

- Rats show a discounting curve on cognitive effort discounting as effort costs increase.
- High dose testosterone treatment does not affect cognitive effort discounting.
- Cognitive effort discounting is sensitive to serotonin, but not dopamine manipulations.

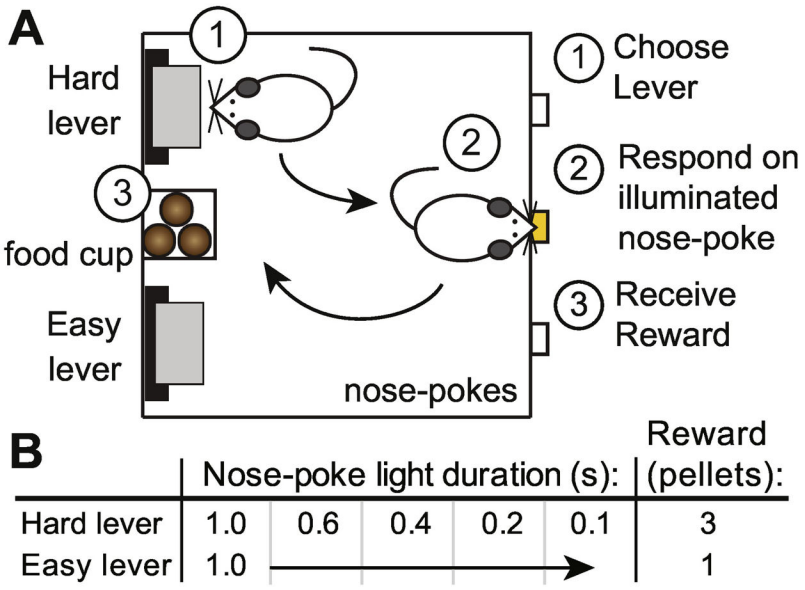

Figure 1.

Schematic of the operant chamber (A) and experimental design (B) for cognitive effort discounting. (A) Response on the lever (1) illuminates a nose-poke light on the opposite wall. The rat must respond in the illuminated nose-poke (2) for a correct trial to deliver a reward in the food cup (3). (B) For choice of the hard lever, the nose-poke light duration decreases across successive blocks of trials from 1.0 s to 0.1 s; the nose-poke light remains constant at 1.0 s with a response on the easy lever. A correct response earns 3 sucrose pellets for a hard lever trial, but only 1 pellet when the easy lever is selected.

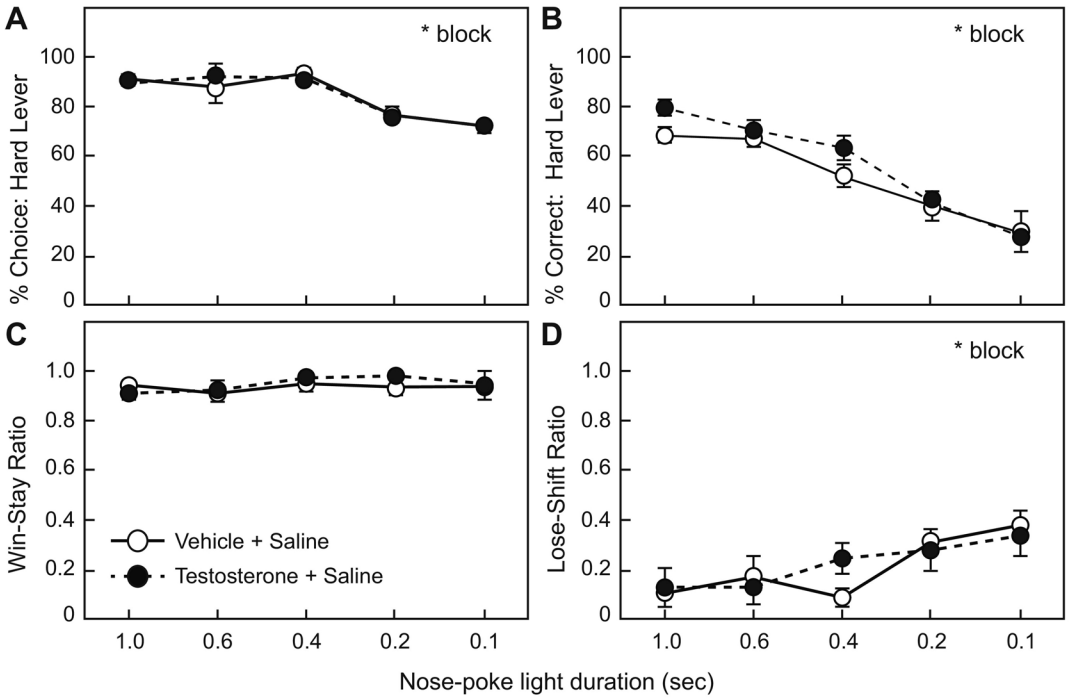

Figure 2.

Hard lever choice (percent choice, A) and task accuracy (percent correct response, B) in vehicle- (open circles, solid line) and testosterone-treated rats (closed circle, dotted line) in an operant model of cognitive effort discounting (see Methods for task details). Rats were pretreated with saline. Win-stay and Lose-shift ratios are shown in C and D. Asterisk indicates significant effect of block (nose-poke light duration) on lever choice, task accuracy, and Lose-shift ratio.

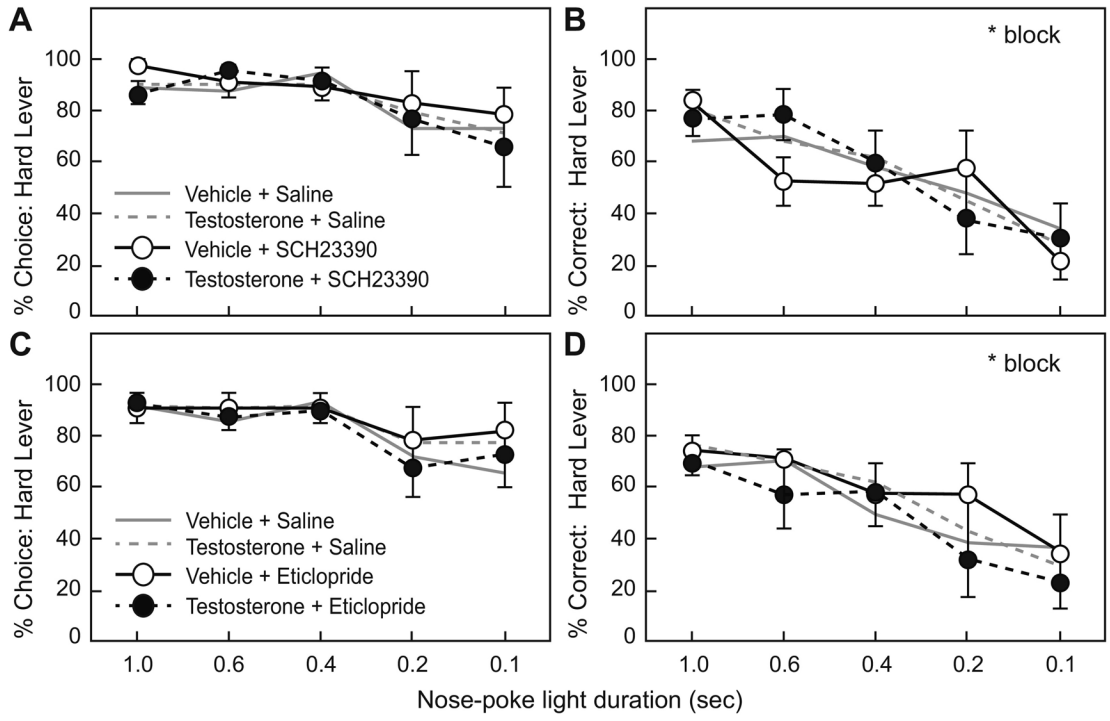

Figure 3.

Hard lever choice (percent choice, A, C) and task accuracy (percent correct response, B, D) in vehicle- (open circles, solid line) and testosterone-treated rats (closed circle, dotted line) in an operant model of cognitive effort discounting (see Methods for task details). Rats were pretreated with the dopamine D1 receptor antagonist SCH-23390 hydrochloride (SCH) at 0.03 mg/kg (A, B), or the D2 receptor antagonist S-(-)-eticlopride hydrochloride (eticlopride) at 0.06 mg/kg (C, D). Baseline saline data are shown in gray for comparison. Asterisk indicates significant effect of block (nose-poke light duration) on task accuracy.

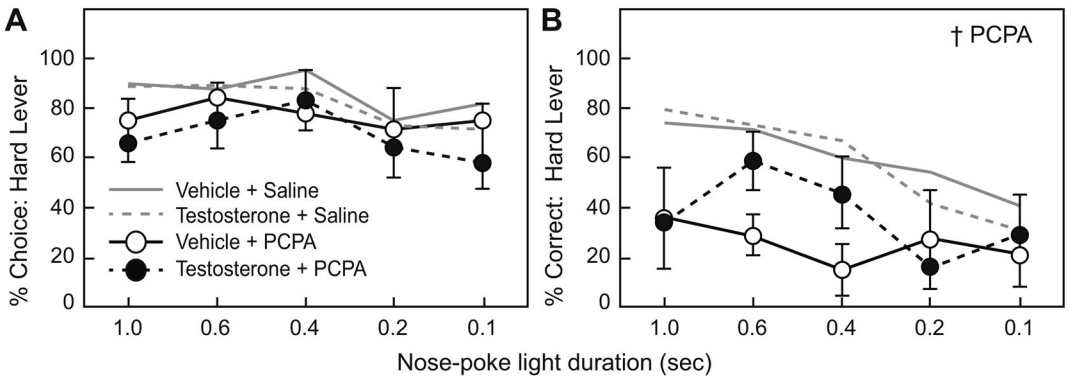

Figure 4.

Hard lever choice (percent choice, A) and task accuracy (percent correct response, B) in vehicle- (open circles, solid line, n=4) and testosterone-treated rats (closed circle, dotted line, n=6) in an operant model of cognitive effort discounting (see Methods for task details). For serotonin depletion, rats were pretreated with 4-chloro-DL-phenylalanine (PCPA, 300 mg/kg) at 24 and 48h before testing. Dagger indicates significant effect of PCPA on task accuracy.