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ANABOLIC-ANDROGENIC STEROIDS IMPAIR SET-SHIFTING AND REVERSAL LEARNING IN MALE RATS

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Abstract

Anabolic-androgenic steroid (AAS) abuse is prevalent not only among elite athletes, but is increasingly common in high school and collegiate sports. AAS are implicated in maladaptive behaviors such as increased aggression and risk taking, which may result from impaired cognition. Because they affect dopamine function in prefrontal cortical (PFC)-striatal circuitry, AAS may disrupt PFC-dependent processes such as behavioral flexibility. This was the focus of the present study. Adolescent male Long-Evans rats were treated chronically with high-dose testosterone (7.5 mg/kg in water with 13% cyclodextrin) or vehicle sc, and tested for set-shifting and reversal-learning. For set-shifting, rats were trained on a visual cue task (VCT), then were shifted to a direction cue task (DCT), or vice-versa. For reversal learning, rats were first trained on VCT and were then required to press the opposite lever. 2-cue set-shifting introduced a novel paradigm in which rats shifted from a 1-Light Visual Task (1LVT) to a tone cue task (TCT). Testosterone-treated rats were significantly impaired on the set-shift from DCT to VCT compared to vehicle-treated controls (trials to criterion: vehicle 240.9±29.9, testosterone 388.3±59.3, $p<0.05$). However, on the set-shift from VCT to DCT, testosterone did not affect performance. During reversal-learning, testosterone significantly increased trials to criterion (vehicle: 495.9±91.8 trials, testosterone: 793.7±96.7 trials, $p<0.05$). In 2-cue set-shifting, testosterone diminished performance and the difference showed borderline significance (vehicle: 443.2±84.4 trials, testosterone: 800.4±178.2 trials, $p=0.09$). Our results show that testosterone impairs behavioral flexibility and have implications for understanding cognitive and 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. RIW advised throughout the study and edited the manuscript. All authors have approved the final manuscript.

AUTHOR DISCLOSURES

All authors declare they have no conflicts of interest.

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Keywords

anabolic agents; cognition; operant behavior; food reward; testosterone

INTRODUCTION

Anabolic-androgenic steroids (AAS) are drugs of abuse used to increase muscle mass and enhance athletic performance. Once restricted to elite athletes, AAS abuse is now present in high school and college athletics. In fact, 4–6% of high school males admit using AAS (Yesalis and Bahrke, 2005). Health risks of AAS include cardiovascular, hepatic and reproductive dysfunction (Pope *et al*, 2013). AAS may also cause maladaptive behavioral changes, including increased impulsivity and aggression (Wood *et al*, 2013). Recent human studies further suggest that AAS induce cognitive impairments. AAS users exhibited diminished visuospatial memory compared to non-users, and the level of impairment was correlated with lifetime AAS use (Kanayama *et al*, 2012). However, little is known about the effects of AAS on other aspects of cognition, including behavioral flexibility. Behavioral flexibility allows appropriate adaptations in dynamic environments. The present study determined if AAS impair behavioral flexibility. AAS have been implicated in changes to dopamine function in the prefrontal cortical-striatal circuitry on which behavioral flexibility depends (Wood *et al*, 2013; Kurling-Kailanto *et al*, 2010). In particular, because prefrontal cortex (PFC) circuitry is still developing during adolescence (Blakemore and Choudhury, 2006), it is important to understand how adolescent steroid use may impair behavioral flexibility and its underlying neurobiological mechanisms.

Studies of AAS effects in humans are complicated by users' motivation to increase muscle-mass and enhance appearance. Animal studies eliminate this confound, and also control for AAS type and dose. The present study exposed male rats to chronic high-dose testosterone beginning in adolescence. This is relevant to patterns of AAS abuse in humans, as the majority of users are male and begin using steroids as teenagers or young adults. Among American high school students, 4.3% of men have used AAS compared to 2.2% of women (Johnston *et al*, 2013). Rats in the present study received testosterone because it is the prototypical AAS. All AAS are derived from testosterone, and testosterone itself is a popular choice among human users. Testosterone was the most-common (55.5%) 'adverse analytical finding' in urine tests at World Anti-Doping Agency-accredited laboratories during 2011 (WADA, 2012).

Behavioral flexibility is tested in animals using adaptations of human paradigms. In both humans and animals, subjects first learn stimulus-response rules to earn reward. When the rules change, subjects must shift response strategies to continue earning reward. Set-shifting is evaluated in humans by the Wisconsin Card Sorting Task (Berg, 1948), and in rats by shifts from one discrimination task to another in a maze or operant chamber (Floresco *et al*, 2008). Reversal learning is the ability to respond to a simple inversion of a rule. (Ghods-Sharifi *et al*, 2008).

A considerable literature on the role of various brain regions, neurotransmitters, and environmental stimuli in set-shifting and reversal learning has accumulated in recent years.

Performance on set-shifting and reversal learning tasks is sensitive to lesions of PFC and dopamine (DA) manipulations in the mesocorticolimbic circuitry (Ghods-Sharifi *et al*, 2008; Floresco *et al*, 2006, 2008). Set-shifting depends on function of the medial PFC (mPFC), while reversal learning is dependent on orbital PFC (OFC) activity (Floresco *et al*, 2008; Floresco, 2013; McAlonan & Brown, 2003). DA release increases in mPFC and nucleus accumbens (Acb) during set-shifting and reversal learning (Stefani and Moghaddam, 2006; van der Meulen, 2007). Castration decreases dopaminergic afferents to the mPFC in male rats, and this decrease is attenuated by androgen replacement (Kritzer, 2000). This suggests that the dopaminergic circuitry on which behavioral flexibility depends is sensitive to steroid hormones. Set-shifting and reversal learning are also sensitive to DA function within subcortical brain regions. Manipulations of DA receptors in mPFC and Acb impair set-shifting and reversal learning (Floresco and Magyar, 2006; Haluk and Floresco, 2009). AAS alter dopamine receptor density in Acb, suggesting another possible mechanism for testosterone to affect behavioral flexibility (Kindlundh *et al*, 2001).

Deficits in behavioral flexibility often result from an increase in perseverative behavior, the inability to cease use of a response strategy when it is no longer relevant. Perseveration has been associated with high levels of testosterone in both humans and animals. In a foraging paradigm, male chicks treated with testosterone continually peck grains of only one preferred color, while vehicle-treated chicks peck both grain colors (Andrew and Rogers, 1972). In humans, adolescent males exhibiting external signs of high testosterone are better at performing simple repetitive tasks than their peers with low testosterone, independent of cognitive ability (Broverman *et al*, 1964). We hypothesized that chronic exposure to AAS would increase perseveration and impair set-shifting and reversal learning in rats.

EXPERIMENTAL PROCEDURES

Animals

Adolescent male Long-Evans rats (5 weeks of age at the start, Charles River Laboratories, MA) were pair-housed under a reversed 14L:10D photoperiod. They remained gonad-intact to approximate human AAS use. Behavior was tested during the first 4 hours of the dark phase. To facilitate operant responding, rats were maintained on a slow rate of growth (3–4 g/day) as in our previous studies (Cooper *et al*, 2014). 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).

AAS treatment

For at least 2 weeks before behavioral training and throughout the study, rats (n=10–12/group) received injections 5 d/week of testosterone (7.5 mg/kg; Steraloids, RI) or aqueous vehicle [3% ethanol and 13% cyclodextrin (RBI, MA)] sc. This dose approximates heavy steroid use in humans, and has been used previously to demonstrate AAS effects on behavior in rats (Clark *et al*, 1998; Clark and Fast, 1996; Wood *et al*, 2013).

Operant Chambers

Testing was conducted in operant chambers (Med Associates, VT), enclosed in sound-attenuating boxes with fans for ventilation. Chambers had 2 retractable levers with stimulus lights flanking a pellet dispenser with food cup (Figure 1). For 2-cue Set-Shifting, chambers were fitted with an audible tone cue and a single stimulus light between the levers.

Experimental Design

Set-shifting—Rats were tested for strategy set-shifting from the Visual Cue Task (VCT) to the Direction Cue Task (DCT; VCT→DCT) according to modification of Floresco *et al* (2008). Additional groups of testosterone- and vehicle-treated rats were tested for set-shifting from DCT to VCT (DCT→VCT).

Reversal learning—Testosterone- and vehicle-treated rats were tested for reversal learning on the VCT.

2-cue set-shifting—While the initial set-shifting experiment evaluated the shift between an external sensory cue (VCT) and an internal position cue (DCT), 2-cue set-shifting tested the shift between two external sensory cues (visual and auditory). Testosterone- and vehicle-treated rats were tested for set-shifting from the 1-Light Visual Task (1LVT) to the Tone Cue Task (TCT; 1LVT→TCT).

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 in daily sessions of 90 trials. Each 20-second trial began in darkness with both levers retracted in the inter-trial interval (ITI) state. 3 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 90 trials. Stimulus lights were never illuminated during training.

Side bias was determined on the final day of training before testing for acquisition of VCT or DCT. Both levers were inserted on each trial. When the rat responded on 1 lever, both levers retracted, and 1 pellet was delivered. On subsequent trials, the rat was only rewarded for responding on the opposite lever, and trials continued until the rat responded on both levers. This was repeated 7 times. The lever selected first on 4 of 7 sessions was considered the side bias.

Testing

Rats were tested in daily sessions of 120 trials (20 seconds/trial). For set-shifting and reversal learning, a trial began with illumination of 1 stimulus light. In a pair of trials, the left and right stimulus lights were each illuminated once in random order. 3 seconds later, both levers were inserted and the house-light was illuminated. A correct response resulted in

immediate delivery of 1 pellet and retraction of the levers. The house-light remained illuminated for 4 seconds before ITI. With an incorrect response or trial omission, the chamber immediately reverted to ITI. For VCT, the rat was rewarded for a response on the lever under the illuminated light (Figure 1A). For VCT reversal learning, rats were required to respond on the lever under the darkened stimulus light (Figure 1B). For DCT, a stimulus light was illuminated, but rats were required to ignore the light and respond on the same lever (either left or right) on every trial (Figure 1C). Rats were assigned to the lever opposite to their side bias. For 2-cue set-shifting, trials began with activation of the stimulus light and/or tone. In 1LVT, rats were required to ignore the tone and to respond on the right lever when the light was illuminated, and on the left lever when it was darkened (Figure 1D). For TCT, rats had to ignore the light and to respond on the left lever when the tone was on, and on the right lever when the tone was off (Figure 1E).

Performance—For set-shifting, criteria for success on the VCT and DCT during acquisition and set-shifting are from Floresco *et al* (2008). For acquisition, rats were required to make 8 consecutive correct responses, with a minimum of 30 trials. During set-shifting, rats were required to make 10 consecutive correct responses. For reversal learning and 2-cue set-shifting, criteria for success during both acquisition and shift/reversal was 8 consecutive correct responses. Error analysis was conducted as in Floresco *et al* (2008). *Perseverative* errors occurred when the rat made a response that was correct on the previous task. For example, in VCT→DCT a perseverative error occurred when the rat chose the left lever with an illuminated stimulus light, when he was required to respond on the right lever. Sessions were divided into blocks of 16 trials. After making <5 perseverative errors in any block, subsequent errors of this type were designated as *regressive* errors. *Never-reinforced* errors were responses that would not be correct in either task (e.g. the rat chose the left lever when the right stimulus light was illuminated and DCT required response on the right lever).

Data Analysis

For each experiment (set-shifting, reversal learning, and 2-cue set-shifting), the number of trials required to reach criterion on each task by vehicle- and testosterone-treated groups was compared by repeated measures (RM)-ANOVA, with task (acquisition and shift/reversal) as the repeated measure. When there was a main effect of drug treatment or interaction of drug x task, performance of vehicle- and testosterone-treated groups was compared on each task by Student's t-test. For each shift/reversal, errors were averaged for testosterone- and vehicle-treated groups. Total errors for the two groups were compared by Student's t-test, and error subtypes (perseverative, regressive, and never-reinforced errors) were compared by RM-ANOVA.

RESULTS

Set-Shifting

Figure 2 presents trials to criterion (mean±SEM) during acquisition and set-shift for DCT→VCT and VCT→DCT. There was no effect of testosterone on initial acquisition of either DCT or VCT ($p>0.05$). In DCT→VCT, there was a significant drug x task interaction ($F_{1,18}=5.7$, $p<0.05$ by RM-ANOVA). Testosterone-treated rats were impaired on set-

shifting, requiring significantly more trials to reach criterion in the shift to VCT than vehicle controls (vehicle: 240.9 ± 29.9 trials, testosterone: 388.3 ± 59.3 trials, $p < 0.05$). Testosterone-treated rats also made significantly more errors during the shift to VCT (vehicle: 99.4 ± 12.3 , testosterone: 172.9 ± 30.7 , $p < 0.05$). However, testosterone did not selectively increase any specific type of error (Table 1). In VCT→DCT testosterone did not increase trials to criterion during the set-shift or errors committed in the shift to DCT ($p > 0.05$) (Figure 2B).

Reversal Learning

Figure 3 presents trials to criterion (mean±SEM) during acquisition and reversal of VCT. Testosterone did not impair the initial acquisition of VCT ($p > 0.05$). By RM-ANOVA, there was a significant drug x task interaction ($F_{1,17}=4.7$, $p < 0.05$). Testosterone-treated rats were impaired on reversal learning compared to vehicle-treated rats, indicated by an increased number of trials required to reach criterion (vehicle: 495.9 ± 91.8 trials, testosterone: 793.7 ± 96.7 trials, $p < 0.05$). However, errors by testosterone-treated rats were not significantly different from controls ($p > 0.05$).

2-cue Set-Shifting

Figure 4 presents trials to criterion (mean±SEM) during acquisition and set-shift for 1LVT→TCT. Testosterone did not impair acquisition of 1LVT ($p > 0.05$). However, by RM-ANOVA, there was a main effect of drug treatment, with testosterone significantly increasing trials to criterion ($F_{1,17}=4.7$, $p < 0.05$). Similar to results on DCT→VCT, testosterone impaired performance on the set-shift to TCT. Testosterone-treated rats required trials 800.4 ± 178.2 trials to reach criterion, vs 443.2 ± 84.4 for vehicle-treated controls, and this difference showed borderline significance ($p = 0.09$ by Student's t-test).

DISCUSSION

The present study investigated the effects of chronic high-dose testosterone treatment on two aspects of behavioral flexibility: set-shifting and reversal learning. As hypothesized, testosterone impaired set-shifting in DCT→VCT by increasing the number of trials to criterion and the number of errors compared to vehicle controls. However, in VCT→DCT, testosterone- and vehicle-treated groups performed equally well on both the initial task acquisition and set-shift. Testosterone also diminished reversal learning for VCT. Finally, in 2-cue set-shifting, testosterone impaired performance and increased trials to criterion. Importantly, testosterone never affected initial acquisition; it only impacted flexibility (shifting or reversal performance). These results indicate that high-dose testosterone treatment impairs behavioral flexibility without causing a general learning deficit during task acquisition.

Behavioral flexibility is dependent on the PFC and dopaminergic function in the mesocorticolimbic DA system (Birrell and Brown, 2000; Haluk and Floresco, 2009). AAS affect DA function within the mesocorticolimbic circuitry, providing a possible mechanism for these drugs to impair behavioral flexibility (Birgner et al, 2007; Kindlundh et al, 2001; Kurling-Kailanto et al, 2010). The mesocorticolimbic DA system involves dopaminergic projections from the midbrain to the PFC and striatum (Kauer and Malenka, 2007).

Dopamine efflux in the PFC increases during set-shifting and reversal learning, suggesting that dopaminergic projections to the PFC are important for behavioral flexibility (Stefani and Moghaddam; van der Meulen, 2007). Furthermore, specific aspects of behavioral flexibility depend on subregions of the PFC including the orbital frontal cortex (OFC) and medial prefrontal cortex (mPFC) (Floresco, 2013). Behavioral flexibility is not only dependent on PFC function, but is also sensitive to DA manipulations within the dorsal striatum and ventral striatum (nucleus accumbens, Acb) (Clarke et al, 2011; Haluk and Floresco, 2009; O'Neill and Brown, 2007).

The decreased set-shifting ability of testosterone-treated rats in DCT→VCT and 2-cue set-shifting supports our hypothesis that testosterone reduces behavioral flexibility. Set-shifting is a complex aspect of behavioral flexibility requiring rats to perform an attentional shift from one stimulus dimension to another. When the rules change, they must learn to both ignore the previously-relevant stimulus (i.e light) and to attend the newly-relevant stimulus (i.e direction). Within the PFC, lesions or inactivation of the medial prefrontal cortex (mPFC) impair set-shifting and increase perseveration (Floresco *et al*, 2008). Set-shifting is also exquisitely dependent on normal DA receptor balance within the Acb. Administration of either a D1 receptor antagonist or D2 receptor agonist within the Acb decrease set-shifting performance (Haluk and Floresco, 2009). Like testosterone-treatment in this study, D1 receptor antagonism in Acb impaired set-shifting without increasing perseverative errors. Similar to application of a D1 receptor antagonist and D2 receptor agonist, high doses of testosterone have been shown to decrease D1 while increasing D2 receptor density in Acb (Kindlundh *et al*, 2001). Thus, the effects of testosterone on D1/D2 receptor balance in Acb could be one mechanism by which AAS diminish set-shifting performance.

Testosterone's impairment of reversal learning further supports our hypothesis that AAS interfere with behavioral flexibility. While set-shifting requires rats to shift attention between different stimulus dimensions, reversal learning requires rats to respond to a simple inversion of a rule. In contrast to set-shifting, which depends on mPFC function, reversal learning is disrupted by inactivation of the orbital frontal cortex (OFC), but not mPFC (Floresco, 2013; McAlonan & Brown, 2003). In humans, high levels of endogenous testosterone correlate with decreased OFC activity, suggesting a mechanism for testosterone to impair reversal learning (Mehta and Beer, 2010). Taken together, impairment of both set-shifting and reversal learning suggest testosterone may have broad effects on multiple PFC regions.

Like set-shifting, reversal learning is sensitive to DA manipulations in subcortical regions. Parkinson's disease patients with depleted striatal DA have diminished behavioral flexibility, and striatal DA depletion in rats and primates impairs reversal learning (Clarke et al, 2011; Monchi *et al*, 2004; O'Neill and Brown, 2007). Likewise, we have recently shown that high-dose testosterone decreased striatal levels of tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis (Wood *et al*, 2013). Thus, testosterone may impair reversal learning by decreasing striatal DA or modulating Acb DA receptor density. Like set-shifting, reversal learning is also sensitive to DA receptor manipulation in Acb. However, reversal learning is only impaired by D2 receptor agonism in Acb, and is unaffected by D1 antagonists (Haluk

and Floresco, 2009). Therefore, set-shifting and reversal learning depend on specific aspects of mesocorticolimbic DA function, and these aspects are susceptible to AAS modulation.

While testosterone-treated rats exhibited set-shifting deficits in DCT→VCT, the lack of testosterone effect in VCT→DCT was surprising. As applied in the present study, DCT may not be useful for testing behavioral flexibility in testosterone-treated adolescent rats due to a floor effect. During acquisition and set-shifting, rats mastered the DCT task significantly more quickly than VCT. Rats required about twice as many trials to acquire VCT than to acquire DCT, and almost six times more trials to set-shift to VCT compared to DCT. Thus, in contrast to previous work by Floresco et al (2008), DCT was easier than VCT for rats in this study. Also compared to Floresco et al (2008), control rats in the present study required more days to complete both set-shifting and reversal learning paradigms. These differences may be explained by cognitive differences between adults and the adolescent rats used in the current study. In this regard, adolescent rats may exhibit less baseline behavioral flexibility than adults, but this remains to be tested. The difference in performance on VCT and DCT may also be explained by this developmental difference: DCT requires simple, repetitive response behavior that may actually be enhanced by adolescence (Spear and Brake, 1983). Thus, VCT may be an easier task for adult rats, while DCT is easier for adolescents.

However, we could not exclude the possibility that testosterone may selectively impair attention to external sensory stimuli. If so, set-shifting to VCT would be more difficult for testosterone-treated rats. This is unlikely, as testosterone-treated rats were never impaired on initial acquisition of VCT, suggesting their visual discrimination learning was intact. Nevertheless, to test this potential confound, we developed the 2-cue set-shifting experiment in which both tasks require attention to external sensory stimuli. 2-cue set-shifting was more difficult than previous set-shifting tasks, as all rats required more trials to reach criterion on 1LVT and TCT than on VCT and DCT. In 2-cue set-shifting testosterone once again impaired performance on the shift without affecting initial acquisition. This result confirms that testosterone decreased behavioral flexibility without affecting attention to sensory stimuli or general learning ability.

This study confirms that AAS decrease performance in multiple aspects of behavioral flexibility including both set-shifting and reversal learning. These behavioral impairments correspond to known effects of AAS on brain anatomy and physiology, as these drugs affect DA function in relevant regions of the mesocorticolimbic DA system. Specifically, AAS decrease DA levels in the dorsal striatum and cause DA receptor imbalance in the Acb (Kindlundh *et al*, 2001; Wood *et al*, 2013). These manipulations of the DA system are known to impair behavioral flexibility and other aspects of executive function such as decision making (Clarke *et al*, 2011; Haluk and Floresco, 2009; O'Neill and Brown, 2007).

Questions remain regarding the mechanisms through which AAS modify behavioral flexibility. In the current study, high-dose testosterone was injected daily before behavioral testing. Thus, testosterone may exert its effects both via chronic actions on classical genomic receptors and rapidly via non-genomic receptors (Sato *et al*, 2008). We also cannot differentiate whether testosterone is exerting its effects through androgen receptors or estrogen receptors, since testosterone can be converted in the brain to androgenic or

estrogenic metabolites. Commonly-abused AAS include both non-aromatizable androgens such as stanozolol, as well as methandrostenolone (Dianabol) and other aromatizable androgens. However, our studies of AAS self-administration suggest that the reinforcing effects are related to androgenic potency (DiMeo and Wood, 2006). This suggests that AAS effects on Acb are, at least in part, acting via androgenic mechanisms. Finally, this study investigated the effects of testosterone at supraphysiologic levels as a model of human AAS use, but our findings do not necessarily translate to the effects of testosterone within the normal physiologic range. In this regard, a previous study by Kritzer *et al* (2007) found that castration impaired flexibility in male rats, and that this deficit was rescued by administration of testosterone propionate at physiologic levels. Thus, it seems that a normal level of testosterone facilitates behavioral flexibility, and that very low or very high levels impair performance.

The present study has disturbing implications for human steroid users. Our results suggest AAS use diminishes behavioral flexibility—the ability to update behavior according to the fluctuating situational demands of daily life in dynamic human environments. Furthermore, many users initiate AAS use as adolescents, when the PFC circuitry underlying executive function is still developing (Blakemore and Choudhury, 2006). Thus, AAS use may be particularly dangerous for adolescents, exacerbating adolescent deficits in executive function. Understanding cognitive effects of AAS will inform the public and health professionals of previously unknown risks of this drug.

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Figure 1.

Operant tasks. A) For the Visual Cue Task (VCT), rats respond on the lever with an illuminated stimulus light to receive food reward. B) For the VCT Reversal, rats respond on the lever with the darkened light. C) For the Direction Cue Task (DCT), rats respond on the same lever in every trial (left or right) regardless of stimulus light. D) For 1-Light Visual Task (1LVT) rats respond on the right lever when the stimulus light is on, and the left lever when the light is off. E) In the Tone Cue Task (TCT), rats respond on the left lever when the tone is on, and on the right lever when the tone is off.

Figure 2.

Trials to criterion (mean±SEM) during acquisition and set-shifting, and errors committed (mean±SEM) during the set-shift in testosterone- (dark bars) and vehicle-treated rats (light bars). A) Direction Cue Task (DCT, striped bars) acquisition and set-shift to Visual Cue Task (VCT, solid bars). B) VCT acquisition and set-shift to DCT. Asterisk indicates $p < 0.05$ by Student's t-test. Testo = Testosterone.

Figure 3.

Trials to criterion (mean±SEM) during acquisition and reversal of VCT, and errors committed (mean±SEM) during the VCT Reversal in testosterone- (dark bars) and vehicle-treated rats (light bars). Asterisk indicates $p < 0.05$ by Student's t-test.

Figure 4.

Trials to criterion (mean±SEM) during acquisition of 1LVT and set-shift to TCT, and errors committed (mean±SEM) during the set-shift in testosterone- (dark bars) and vehicle-treated rats (light bars). Cross indicates main effect of drug by RM-ANOVA. Testo = Testosterone.

Table 1

Mean $\pm$ (SEM) errors of each type committed by vehicle and testosterone-treated groups during set-shifting to VCT and DCT. Testo = Testosterone.

Set-shifting Errors by Type				
Task	Group	Perseverative Errors	Regressive Errors	Never-Reinforced Errors
Set-Shift to VCT	Vehicle	64.1 (15.6)	24.5 (10.3)	10.8 (2.6)
	Testo	83.5 (28.4)	65.2 (22.6)	24.2 (9.8)
Set-Shift to DCT	Vehicle	8.8 (1.8)	6.4 (2.3)	4.8 (1.2)
	Testo	8.3 (1.7)	4.7 (2.1)	3.0 (0.5)