European Neuropsychopharmacology (2015) **25**, 583–590

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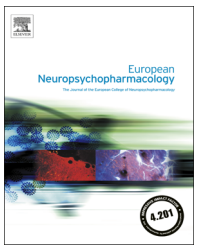

Anabolic-androgenic steroids impair set-shifting and reversal learning in male rats

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Received 8 October 2014; received in revised form 18 December 2014; accepted 8 January 2015

KEYWORDS
Anabolic agents;
Cognition;
Operant behavior;
Food reward;
Testosterone

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.

1. Introduction

Anabolic-androgenic steroids (AAS) are drugs of abuse used to increase muscle mass and enhance athletic performance.

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http://dx.doi.org/10.1016/j.euroneuro.2015.01.002
0924-977X/

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 and Brown, 2003). DA release increases in mPFC and nucleus accumbens (Acb) during set-shifting and reversal learning (Stefani and Moghaddam, 2006; van der Meulen et al., 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.

2. Experimental procedures

2.1. 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 h 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 (US) (2011)

2.2. 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).

2.3. 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 (Fig. 1). For 2-cue Set-Shifting, chambers were fitted with an audible tone cue and a single stimulus light between the levers.