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'Roid rage in rats? Testosterone effects on aggressive motivation, impulsivity and tyrosine hydroxylase

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HIGHLIGHTS

- We tested effects of chronic, high-dose testosterone on aggressive motivation and impulsivity in male rats.
- Testosterone did not enhance motivation for aggression, but did increase fighting.
- Testosterone reduced impulsivity in a delay-discounting test for food reward.
- Testosterone selectively reduced levels of tyrosine hydroxylase in caudate-putamen.

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ABSTRACT

In humans and animals, anabolic-androgenic steroids (AAS) increase aggression, but the underlying behavioral mechanisms are unclear. AAS may increase the motivation to fight. Alternatively, AAS may increase impulsive behavior, consistent with the popular image of 'roid rage. To test this, adolescent male rats were treated chronically with testosterone (7.5 mg/kg) or vehicle and tested for aggressive motivation and impulsivity. Rats were trained to respond on a nose-poke on a 10 min fixed-interval schedule for the opportunity to fight in their home cage with an unfamiliar rat. Although testosterone increased aggression (6.3 ± 1.3 fights/5 min vs 2.4 ± 0.8 for controls, $p<0.05$), there was no difference in operant responding (28.4 ± 1.6 nose-pokes/10 min for testosterone, 32.4 ± 7.0 for vehicle). This suggests that testosterone does not enhance motivation for aggression. To test for impulsivity, rats were trained to respond for food in a delay-discounting procedure. In an operant chamber, one lever delivered one food pellet immediately, the other lever gave 4 pellets after a delay (0, 15, 30 or 45 s). In testosterone- and vehicle-treated rats, body weights and food intake did not differ. However, testosterone-treated rats chose the larger, delayed reward more often (4.5 ± 0.7 times in 10 trials with 45 s delay) than vehicle controls (2.5 ± 0.5 times, $p<0.05$), consistent with a reduction in impulsive choice. Thus, although chronic high-dose testosterone enhances aggression, this does not include an increase in impulsive behavior or motivation to fight. This is further supported by measurement of tyrosine hydroxylase (TH) by Western immunoblot analysis in brain regions important for motivation (nucleus accumbens, Acb) and executive function (medial prefrontal cortex, PFC). There were no differences in TH between testosterone- and vehicle-treated rats in Acb or PFC. However, testosterone significantly reduced TH (to $76.9 \pm 3.1\%$ of controls, $p<0.05$) in the caudate-putamen, a brain area important for behavioral inhibition, motor control and habit learning.

1. Introduction

Anabolic-androgenic steroids (AAS) are performance-enhancing substances. Misuse of AAS by athletes is widely acknowledged, but potential health risks are not well-understood. These include not only cardiovascular, hepatic and reproductive dysfunction, but also alterations in brain and behavior. Many AAS users meet DSM criteria for psychoactive substance dependence, including continued use despite negative side effects, and withdrawal symptoms when steroids are discontinued [1]. However, unlike other illicit drugs, AAS have only a limited capacity to cause acute intoxication or other immediate physiologic responses [2]. Instead, a potential danger of AAS abuse reflects the increased likelihood that users will engage in behaviors that pose significant risks to themselves and others. Steroid use has been implicated in several violent murders [3–6]. Similarly, in surveys of current users and in prospective studies of human volunteers, increased aggression is the most consistent behavioral effect of high-dose AAS exposure in humans [7–16]. This has given rise to the image of "roid rage": a sudden and exaggerated aggressive response to a minimal

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provocation. Roid rage is recognized in popular media [17], in body-building circles [7,18,19], and in clinical literature [3–6].

Investigating AAS use in humans is complicated by the user's motivation for increased strength and muscle mass [1,2]. Animal studies can explore consequences of AAS in an experimental context where appearance and athletic performance are irrelevant. Such studies show that AAS are rewarding, as demonstrated by self-administration [20–29] and conditioned place preference (CPP) [30–37]. Furthermore, AAS stimulate social behavior, particularly mating and aggression [20,38–42]. In a resident–intruder test, attack latency by the steroid-treated home-cage male is reduced, and the composite aggression score increases [40]. With additional stimulation (tail pinch), steroid-treated males will attack anestrous females [41,42].

The present study investigated potential underlying causes of AAS-induced aggression in rats, including increases in impulsivity and aggressive motivation. Loss of impulse control is one of the generally-accepted, but relatively untested, features of roid rage. According to this model, a small provocation (introduction of an intruder into the home cage) in a rat treated with AAS would produce an exaggerated behavioral response (short-latency attack). Alternatively, androgens may enhance aggressive motivation. Agonistic behavior is rewarding when you win. Animals will work for the opportunity to attack an intruder [43,44], and show CPP for an environment where they previously won fights [45]. Since androgens promote fighting and fighting is rewarding, it is reasonable to expect that chronic exposure to AAS will enhance both aggressive behavior (as demonstrated previously [39,46]) and aggressive motivation. Expression of agonistic behavior involves dopamine (DA) release in the hypothalamus [47]. Winning a fight is also accompanied by DA release from midbrain neurons of the ventral tegmental area (VTA) that project to the nucleus accumbens (Acb) [43,44,46,48]. Adjacent to VTA, dopaminergic neurons in substantia nigra (SN) that project to the caudate-putamen (CPu) coordinate motor control [49]. DA also contributes to executive function in the medial prefrontal cortex (PFC) [50]. Accordingly, the present study used male rats as a model to test the hypothesis that chronic exposure to AAS increases motivation for aggression and enhances impulsive behavior, and that these effects are mediated by increased DA activity in Acb, CPu, PFC, and VTA/SN.

2. Materials and methods

2.1. Animals

Adolescent male Long–Evans rats (4 weeks of age, ca. 75 g BW at the start of the study, Charles River Laboratories, MA) were individually housed under a reversed 12L:12D photoperiod. They remained gonad-intact to approximate AAS use in humans. Behavior was tested under dim red light during the first 4 h of the dark phase when activity peaks.

2.2. AAS treatment

Beginning at 5 weeks, rats received testosterone (7.5 mg/kg; Steraloids, RI) or vehicle [3% ethanol and 13% cyclodextrin (RBI, MA) in water] by daily sc injection 5 days/week (n = 8/group). The 7.5 mg/kg dose approximates a heavy steroid dose in humans, and has been used previously to demonstrate AAS effects on mating and aggression in rats [51,52].

2.3. Aggressive motivation

Methods to test motivation for agonistic behavior are modified from Fish et al. [43]. From 7 weeks of age, males were trained in daily sessions to respond on a nose-poke for the opportunity to fight. An operant conditioning panel containing a nose-poke with stimulus light (Med Associates, VT) was introduced into the home cage 10 min after injection of testosterone or vehicle. A gonad-intact male intruder of similar age and weight was present behind a perforated Plexiglas screen adjacent to the nose-poke, permitting transmission of visual, auditory and olfactory stimuli. Responses on the nose-poke were recorded and reinforced on a fixed-interval (FI) schedule, with introduction of the intruder for 5 min as the reinforcer. Initially, the intruder was introduced 30 s after the start of the session. Subsequently, the FI was increased to 1 min, and by 1-min increments thereafter until a 10 min FI was reached. Intruders were rotated daily, with each test male exposed to the same intruder once every 3 weeks. Using the resident–intruder model ensured that all home-cage rats (testosterone- or vehicle-treated) were dominant to intruders [53].

Testing on the FI10 schedule continued until response rates stabilized (ca. 95 days of age). At this point, aggressive behavior was recorded on videotape during presentation of the intruder male, and was scored by an observer blinded to the treatment groups. Measures of offensive aggression include the number of rolling fights, as well as the latency to the first rolling fight. In addition, we tracked contact with the intruder male in seconds (investigation, threats and dominance displays, aggression), as well as self-grooming and exploration of the cage. The total duration of contact, the number of contact bouts, and the duration of each bout were compared.

2.4. Impulsivity

Rats were subsequently trained and tested for impulsivity, measured as operant responding for food by delay-discounting according to Winstanley et al. [54]. Operant chambers were equipped with a house-light and 2 retractable levers with stimulus lights flanking a food trough connected to a pellet dispenser. Initially, rats were trained to respond on both levers for 45-mg food pellets (Bio-Serv Inc., Frenchtown, NJ). Thereafter, they were tested daily in a series of 4 blocks of 12 trials each. Each block began with 1 forced trial on each lever with stimulus light illuminated, presented in a random order. This was followed by 10 choice trials (both levers extended with lights illuminated). During a 70 s trial, the rat must respond within 10 s after the levers are extended. After receiving reinforcement (or after lever retraction in unreinforced trials), each trial was followed by a time-out in the dark with levers retracted. In the first block of trials, one lever delivered 1 pellet; the 2nd lever delivered 4 pellets. In subsequent blocks, an increasing delay (15, 30, or 45 s) was imposed between a response on the 2nd lever and pellet delivery. When the rat made a response on the lever delivering the large reward, the stimulus light over that lever remained illuminated during the delay. The location of the lever for the large reward (to the left or right of the food trough) was balanced among rats to control for side preferences. Preference for the smaller immediate reward over the larger delayed reward is thought to reflect impulsive behavior [55]. Testing lasted 15 days (until rats were ca. 140 days of age); average daily responses for the last 5 days were compared in testosterone- and vehicle-treated rats. Afterwards, 24-h food intake was measured in both groups of rats to determine if testosterone treatment altered food consumption.

2.5. Tyrosine hydroxylase immunoreactivity

At 20 weeks of age, brains were collected 24 h after the final injection of testosterone or vehicle for measurement of TH protein by Western immunoblot. TH is the rate-limiting enzyme in DA synthesis, and DA is a key neurotransmitter for reward. Rats were sacrificed by decapitation. Brains were quickly removed and regions of interest identified using a standard rat brain atlas [56]. Medial prefrontal cortex (PFC), nucleus accumbens (Acb), caudate-putamen (CPu), and ventral tegmental area/substantia nigra (VTA/SN) were rapidly dissected, immediately frozen on dry ice and stored at −80 °C.

TH was measured according to methods of Jakowec et al. [57]. Briefly, tissue samples were homogenized in buffer (25 mM Tris–HCl, pH 7.4;