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Factors influencing aggression toward females by male rats exposed to anabolic androgenic steroids during puberty

Rebecca L. Cunningham, Marilyn Y. McGinnis *

Department of Biology, The University of Texas at San Antonio, San Antonio, TX 78249, USA

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Abstract

Previous results showed that male rats pubertally exposed to anabolic androgenic steroids (AAS) displayed aggression towards females in response to physical provocation. This experiment examined two factors that may modulate AAS-induced behavior towards females: olfactory cues and frustration. Gonadally intact males began one of three AAS treatments at puberty (D40): testosterone propionate (T), stanozolol (S), T+S, or vehicle control. To test for the relevance of olfactory cues in the elicitation of behavior toward females, a hidden neighbor paradigm was used. The proximal stimulus was an ovariectomized (OVX) female, estrogen plus progesterone (E+P) female, or an E+P female with tape-obstructed vagina (OBS). Distal olfactory cues from a hidden neighbor were delivered from a separate cage connected to the testing arena. The vaginally obstructed, sexually receptive female (OBS) was used to determine the effects of frustration on behavior by AAS males. Both sexual and aggressive behaviors were measured. The presence of distal olfactory cues had no effect on either sexual or aggressive behavior. In the presence of E+P and OBS females, all males displayed sex behaviors, not aggression. However, AAS males displayed significantly more aggression towards proximal OVX females than controls. AAS males mounted OBS females significantly more than controls, indicating a persistence of once rewarded behavior. These results suggest (1) proximal cues of the conspecific female are more salient than distal olfactory cues in determining behavior and (2) AAS males display frustration-induced persistence in response to vaginally obstructed receptive females.

Keywords: Persistence; Male sex behavior; Aggression

Introduction

Anabolic androgenic steroid (AAS) use is increasing among adolescent males (Burnett and Kleiman, 1994; DuRant and Escobedo, 1995; Melia et al., 1996; NIDA, 2002), which is a time of major maturational changes in both brain and behavior (Primus and Kellogg, 1990; Sisk et al., 2003). High levels of AAS have been shown to have various behavioral effects. Adolescent AAS users report increased aggressive behavior, depression, and mood disturbances (Burnett and Kleiman, 1994; Dukarm et al., 1996; Olweus et al., 1980, 1988). Notably, it has been reported that AAS users are more violent towards women (Choi and Pope, 1994).

Studies using animal models have examined the effects of various social and environmental conditions on the aggressive behavior of AAS males (Breuer et al., 2001; Farrell and McGinnis, 2003; Farrell and McGinnis, 2004; Harrison et al., 2000; McGinnis et al., 2002; Melloni et al., 1997). For example, it has been found that male rats chronically exposed to AAS were more aggressive towards other male rats, regardless of whether they were the resident or the intruder during aggression testing (Breuer et al., 2001). This enhanced level of AAS-induced aggression has been confirmed in pubertal male rats exposed to AAS throughout puberty (Farrell and McGinnis, 2003).

Previous studies have shown that physical provocation results in aggression in AAS males (Cunningham and McGinnis, 2006; Farrell and McGinnis, 2003; McGinnis, 2004; McGinnis et al., 2002; Wesson and McGinnis, 2006). For example, adult male rats exposed to the AAS testosterone responded more aggressively towards opponent males than controls in response to physical provocation (mild tail pinch). This heightened aggression occurred whether or not the AAS male or the opponent male was tail pinched, suggesting that AAS lowers the threshold to respond aggressively (McGinnis et

* Corresponding author. Fax: +1 210 458 5493.
E-mail address: Marilyn.McGinnis@utsa.edu (M.Y. McGinnis).

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al., 2002). This potentiation of aggression in response to AAS exposure and physical provocation also occurs in pubescent male rats exposed to the AAS, testosterone (Farrell and McGinnis, 2004; Wesson and McGinnis, 2006).

Typically male rats are not aggressive towards either castrated males or females (Blanchard et al., 1984; DeBold and Miczek, 1984). Social cues, such as the gonadal status of the opponent male, normally influence the level of aggression displayed by male rats (Barfield et al., 1972; Christie and Barfield, 1979). AAS exposure, per se, does not induce aggression towards non-threatening stimuli, such as castrated male opponents (McGinnis et al., 2002). However, when AAS males are physically provoked the level of aggression displayed towards castrated males is dramatically increased (McGinnis et al., 2002).

These findings prompted an investigation into whether AAS-treated males are more aggressive towards females. We found that AAS males were more aggressive towards unreceptive OVX females in response to physical provocation (tail pinch). However, when presented with a receptive female they exhibited sexual behavior, whether or not they were physically provoked (Cunningham and McGinnis, 2006).

Since physically provoked AAS males were aggressive only towards ovariectomized females and not receptive females, olfactory cues may play a role in modulating AAS-induced aggression (Cunningham and McGinnis, 2006). Previously it has been shown that odors from females enhance sexual behaviors in male rodents, whereas odors from other males diminish copulatory behaviors (Koyama, 2004). Conversely, it has been shown that female odors decrease aggression, while male odors increase aggression by male rodents (de Almeida and Miczek, 2002; Fish et al., 1999; Kudryavtseva, 1991; Miczek et al., 2002; Mugford and Nowell, 1970).

Another factor that can influence the likelihood of aggressive behaviors is frustration. Frustration may be defined as the prevention of gratification (Amsel, 1990). In both rodents and humans, withholding or delaying an expected reward has been shown to result in either aggression or persistence of previously rewarded behaviors (Dollard et al., 1939). For example, in some animal studies withholding or delaying a food reward increased aggression (Arnone and Dantzer, 1980; Azrin et al., 1966; Cherek and Heistad, 1971; de Almeida and Miczek, 2002; Gallup, 1965; Matzel, 1984; Miczek et al., 2002), whereas in other studies delayed gratification resulted in persistent display of the once rewarded behavior (running for food) (Amsel, 1958; Amsel and Roussel, 1952). In the current study we used prevention of copulation with a receptive female as an expected reward to produce frustration in sexually experienced male rats pubertally exposed to AAS.

The purpose of the present study was twofold: to identify (1) whether proximal cues or distal olfactory cues are more relevant in determining the behavior of AAS males and (2) whether males pubertally exposed to AAS respond with aggression or sexual behavior in response to a frustrating sexual situation. The importance of olfactory cues was examined using a 'hidden neighbor' apparatus to deliver conflicting olfactory stimuli in the presence of a conspecific female. Frustration was induced by exposing sexually experienced male rats to a receptive female conspecific with a tape-obstructed vagina. Additionally, we studied the effects of two widely abused AAS: testosterone and stanozolol (Mottram and George, 2000; Pope and Katz, 1994).

Materials and methods

Animals

All animals were purchased from Charles River Laboratory (Wilmington, MA) and housed in a temperature-controlled room (23 °C) in standard Plexiglas cages (25 × 20 × 18 cm). Gonadally intact male Long Evans rats were received on postnatal day 35, while intact females were in the weight range of 225–250 g. The animals were given ad libitum access to food and water. Lights were maintained on a 12:12 reversed light/dark cycle, with lights off at 1200 h. Experimental procedures were performed in accordance with National Institutes of Health's guidelines for animal care and use.

Anabolic androgenic steroid treatment

Gonadally intact pubescent males were randomly assigned into one of four treatment groups with a total of 8 animals in each group. On postnatal day 40, the time of preputial separation (Korenbrot et al., 1977), exposure to AAS (Sigma, St. Louis, MO) was initiated (see Table 1). Testosterone propionate (T), stanozolol (S), testosterone propionate plus stanozolol (T + S), or vehicle control (polyethylene glycol 200) were subcutaneously injected at a dosage of 5 mg/kg body weight 5 days a week for 9 weeks.

Testosterone and stanozolol were selected for this study because both are commonly abused AAS, but testosterone has been found to increase aggression, whereas stanozolol actually inhibits it (McGinnis, 2004). The AAS dosage of 5 mg/kg is typically employed in AAS studies as it provides approximately ten times the physiological range of testosterone, and is thus more comparable to AAS levels abused by humans (Breuer et al., 2001; Cunningham and McGinnis, 2006; Farrell and McGinnis, 2003; Kochakian, 1993; McGinnis et al., 2002; Wesson and McGinnis, 2006). Also to more accurately mimic human AAS use, behavioral comparisons were made between AAS males and gonadally intact control males.

Experimental design

All males were tested as young adults between days 68 to 103 and during the dark phase (1200 to 1600) of the light cycle under dim red lighting and videotaped. A neutral cage aggression paradigm was employed as described previously (Cunningham and McGinnis, 2006; Farrell and McGinnis, 2004; McGinnis et al., 2002; Wesson and McGinnis, 2006). All males received sexual experience prior to behavioral testing. Males were placed with receptive females until they achieved an ejaculation. Sexual experience testing was terminated after 30 min if the male did not ejaculate. Only the S-treated males failed to ejaculate during this test.

Three types of conspecific females were used during testing to characterize different states of sexual receptivity and to provide proximal cues to the experimental males. Ovariectomized (OVX) females represented sexually unreceptive animals with no olfactory cues resulting from exposure to either estrogen or progesterone. Sexually receptive females received estrogen plus progesterone (E + P). Sexually receptive females with the vagina obstructed via duct tape were employed as a model for frustration. Frustration has been defined as the withholding of expected gratification (Amsel, 1962). This study used copulation with a receptive female as the expected reward for sexually experience males. Both E + P and OBS females displayed proceptive and receptive sexual behaviors towards males, along with proximal olfactory cues resulting from exposure to estrogen and progesterone (Beach, 1976).

The relevance of olfactory cues in eliciting sexual and aggressive behaviors towards conspecific females was assessed using a hidden neighbor olfactory apparatus. This apparatus enabled us to deliver distal olfactory cues originating from another animal (hidden neighbor) to the experimental males while in the