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Research report

Anabolic steroids have long-lasting effects on male social behaviors

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ABSTRACT

Anabolic androgenic steroids (AAS) use by adolescents is steadily increasing. Adolescence involves remodeling of steroid-sensitive neural circuits that mediate social behaviors, and previous studies using animal models document effects of AAS on male social behaviors. The present experiments tested whether AAS have persistent and more pronounced behavioral consequences when drug exposure occurs during adolescence as compared to exposure in adulthood. Male Syrian hamsters were injected daily for 14 days with either vehicle or an AAS cocktail containing testosterone cypionate (2 mg/kg), nandrolone decanoate (2 mg/kg), and boldenone undecylenate (1 mg/kg), either during adolescence (27–41 days of age) or adulthood (63–77 days of age). As adults, subjects were tested two or four weeks after the last injection for either sexual behavior with a receptive female or male-male agonistic behavior in a resident-intruder test. Compared with vehicle-treated males, AAS-treated males, regardless of age of treatment, displayed fewer long intromissions and a significant increase in latency to the first long intromission, indicative of reduced potential to reach sexual satiety. Increased aggression was observed in males exposed to AAS compared with males treated with vehicle, independently of age of AAS treatment. However, unlike hamsters exposed to AAS in adulthood, hamsters exposed to AAS during adolescence did not display any submissive or risk-assessment behaviors up to 4 weeks after discontinuation of AAS treatment. Thus, AAS have long-lasting effects on male sexual and agonistic behaviors, with AAS exposure during adolescence resulting in a more pronounced reduction in submissive behavior compared to AAS exposure in adulthood.

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1. Introduction

Anabolic androgenic steroids (AAS) are synthetic derivatives of testosterone used to enhance athletic performance and physical appearance. AAS also influence mood and behavior, including heightened aggression and increased or decreased libido in humans [1–4]. AAS use by adolescents in the USA appears to be on the rise [5]. The adolescent brain undergoes extensive remodeling and may therefore be particularly vulnerable to long-term adverse consequences of AAS use during this period of development.

Androgens play an important role in the normal pubertal maturation of male social behaviors. Studies using the male Syrian hamster demonstrate that endogenous testicular hormones organize behavioral circuits during adolescence. Removal of the gonads just prior to the onset of puberty leads to long-lasting impairments in steroid-dependent sexual and agonistic behaviors in adulthood. These impairments cannot be readily reversed by testosterone replacement in adulthood [6–8], providing evidence that during puberty, testicular hormones program adult-typical expression of male social behaviors and that the adolescent brain is more responsive than the adult brain to organizational influences of testosterone on behavioral circuits. These findings lead to the prediction that AAS use during adolescence will have more pronounced and potentially longer lasting behavioral effects than AAS use in adulthood.

Previous work in rodent models has examined short- and long-term effects of AAS on male social behaviors. Generally, AAS exposure increases aggression and decreases sexual behaviors in adults, with specific effects depending on the particular compound, dose, and age at treatment [9,10]. AAS treatment of adolescent males leads to acute increases in both sexual and aggressive behaviors [11,12]. Single AAS compounds, such as testosterone, nandrolone, and stanozolol, increase aggressive and reproductive behaviors of male rats exposed to AAS throughout adolescence, and these effects persist for many weeks after treatment is discontinued [13]. In contrast, while adolescent treatment with an AAS cocktail enhances aggressive behavior in male Syrian hamsters, this effect does not persist beyond 3 weeks after discontinuation of treatment [14]. Altogether, these studies show that AAS can result in persistent changes in behavior in both adults and adolescents that outlast the period of drug exposure, but none of them determined

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whether AAS exposure during adolescence had longer lasting or more pronounced behavioral effects than exposure in adulthood.

The goal of these experiments was to directly compare the long-term behavioral consequences of AAS exposure during adolescence and adulthood. Sexual and aggressive behaviors were assessed either two or four weeks after discontinuation of a two-week treatment with AAS or vehicle during adolescence or adulthood. Because the adolescent brain is more responsive than the adult brain to organizational effects of testosterone [7], our prediction was that relative to AAS exposure in adulthood, adolescent AAS exposure would result in more pronounced and persistent effects on male social behaviors.

2. Materials and methods

Eighteen-day-old male Syrian hamsters (*Mesocricetus auratus*) were obtained from Harlan Sprague–Dawley Laboratories (Madison, WI). Upon arrival subjects were housed in groups of eight per cage (polycarbonate, 33 cm × 38 cm × 17 cm) with ad libitum access to food (Telkad Rodent Diet No. 8640, Harlan) and water. Animal colony temperature was maintained at 22 ± 2 °C, and animals were kept on a light–dark cycle of 14:10 L:D (lights off at 1600 h EST). At 25 days of age, animals were singly housed in polycarbonate cages (30.5 cm × 10 cm × 20 cm, for Experiment 1; 33 cm × 38 cm × 17 cm, for Experiment 2). All animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the Michigan State University Institutional Animal Care and Use Committee.

A cocktail of AAS containing 0.8 mg/ml of testosterone cypionate (TC; Sigma–Aldrich, Inc., St. Louis, MO), 0.8 mg/ml of nandrolone decanoate (ND; Sigma–Aldrich, Inc.), and 0.4 mg/ml of boldenone undecylenate (BU; Steraloids, New Port, Rhode Island) was dissolved in 45% 2-hydroxypropyl-β-cyclodextrin in water (Sigma–Aldrich, Inc.). The AAS cocktail was prepared the day before treatment began for each age group, and was stored thereafter at 4 °C. This is the same cocktail used in previous studies performed in our laboratory and others to understand how AAS exposure affects social behaviors [11,15].

Two separate experiments were conducted to compare the long-term effects of AAS on social behavior of males treated in either adolescence or adulthood. The experimental design was identical for the two experiments with the exception of the type of behavioral test that was conducted at the end of each experiment. In Experiment 1, males were tested with a receptive female to assess sexual behavior. In Experiment 2 males were tested with an intruder male to assess agonistic behaviors.

Male hamsters were singly housed at 25 days of age. At 27 days of age, these gonad-intact males were randomly assigned to AAS-treated or vehicle-treated groups. The two groups were further divided into 2 groups that would be treated either in adolescence or adulthood. Finally, these 4 groups were divided again into groups that would be tested for social behavior either 2 or 4 weeks after discontinuation of treatment. Sample sizes for each of these eight groups of subjects were originally 8–10 in Experiment 1 (sexual behavior) and 13–15 in Experiment 2 (agonistic behavior).

Subjects received a daily subcutaneous injection of either the AAS cocktail (2 mg/kg TC, 2 mg/kg ND, 1 mg/kg BU) or vehicle (2-hydroxypropyl-β-cyclodextrin) for 14 consecutive days, either during adolescence (27–41 days of age) or during adulthood (63–77 days of age). The volume of each daily injection was determined after obtaining each subject's body weight. Injection volumes ranged from 0.1 to 0.25 ml over the two-week course of treatment.

Tests for either sexual or aggressive behaviors were conducted either two or four weeks after the last injection (separate groups of subjects at the two time points). For males treated during adolescence, these tests were conducted at either 56 or 70 days of age. For males treated as adults, the behavioral tests were conducted at 92 or 106 days of age. A repeated measures design was not used in order to avoid potential confounds and additional sources of variability resulting from prior social experience. All behavioral interactions were videotaped with a low-light color video camera and were scored by an experimenter blind to treatment group using a computer program that tags each behavioral code with an elapsed time (software generously provided by Dr. Kim Wallen, Emory University, Atlanta, GA). This system allows the calculation of counts, duration, and latency for each behavior.

2.1. Experiment 1: sexual behavior

Males were sexually inexperienced prior to a standardized behavior test with a receptive female. Tests were conducted under red-light illumination between 1700 and 2030 h EST, one-hour after lights-out, as described in previous studies [6,16–18]. Following a 5 min acclimation to the test chamber (a 51 cm × 26 cm × 31.5 cm glass aquarium with a mirror underneath), males interacted with a stimulus female for 15 min. Ovariectomized stimulus females were hormonally primed to induce receptivity with an estradiol benzoate injection (10 μg in 0.05 ml sesame oil) 48 h before, and a progesterone injection (500 μg in 0.1 ml sesame oil) 4 h before behavior testing. Prior to the behavior test with the subject male, stimulus females were tested for receptivity with a sexually experienced stud male.

Anogenital investigation of the female, mounts, intromissions, and ejaculations were scored and quantified as previously described [6]. In intact male Syrian hamsters sexual satiety is usually reached after five to eight ejaculations. Satiety and latency to reach satiety were assessed by quantifying long intromissions. These are defined as multiple robust rhythmic thrusts, lasting about 20–30 s, followed by genital grooming [19,20]. An efficiency rate was determined for each male by dividing the number of ejaculations achieved by the sum of mounts and intromissions.

Data from three males were excluded because seminal vesicles could not be found upon autopsy following behavior testing, indicating abnormally low levels of endogenous testosterone. Data from 5 subjects were excluded because females did not remain receptive throughout the 15 min test. Data from another four males were excluded because of technical difficulties during videotaping of the behavioral tests; therefore the tests could not be scored. Thus, the final group sample sizes in Experiment 1 were: adolescent vehicle-treated tested on P56, $n = 8$; adolescent vehicle-treated tested on P70, $n = 10$; adolescent AAS-treated tested on P56, $n = 7$; adolescent AAS-treated tested on P70, $n = 9$; adult vehicle-treated tested on P92, $n = 8$; adult vehicle-treated tested on P106, $n = 9$; adult AAS-treated tested on P92, $n = 9$; and adult AAS-treated tested on P106, $n = 8$.

2.2. Experiment 2: agonistic behaviors

Males were tested for agonistic behaviors using a resident-intruder paradigm, a commonly used paradigm to assess agonistic behaviors in Syrian hamsters [21–23], either 2 or 4 weeks after discontinuation of AAS or vehicle treatment during adolescence or adulthood. Tests were conducted between 1700 and 2030 h EST in a red-light illuminated room. A riser made by cutting out the bottom part of a polycarbonate cage was placed in the subject's home cage to make the walls of the cage taller. After a 5 min acclimation period, an age- and weight-matched intruder male was placed in the treated male's home cage. Intruder males were purchased from the same vendor and arrived at the same time as the subject males. Ten-min behavior tests were videotaped and scored for aggressive and submissive behaviors as previously described [15]. Behaviors included in the analysis were: (1) aggressive/dominant behaviors, including flank marking behavior, total contact time, attacks, bites, and offensive posturing; and (2) defensive/submissive behaviors, including defensive posturing, tail-up walking, and escape dashes. In addition, stretch-attend, a risk-assessment measure, was defined and scored as an approach made by the subject in which the body is stretched and lowered, the hind legs stay planted and the front legs stretch forward, eyes are on the opponent and the subject is sniffing in the opponent's direction [24]. To assess overall aggressiveness of each subject, a composite aggression score was obtained by summing the number of flank marks, attacks, bites and offensive (threatening) postures, as these behaviors are measures of overt aggression.

Two males treated during adolescence and four males treated during adulthood were excluded from the statistical analyses because they were determined to be outliers by the Dixon outlier test [25]. In addition, two males assigned to the adult vehicle-treated group were not used because their body weights were too low to test with a weight- and age-matched intruder. Data from six other males were excluded because of technical difficulties during videotaping of the behavioral tests, and therefore their tests could not be scored. Thus, final group sample sizes for Experiment 2 were: adolescent vehicle-treated tested on P56, $n = 12$; adolescent vehicle-treated tested on P70, $n = 10$; adolescent AAS-treated tested on P56, $n = 14$; adolescent AAS-treated tested on P70, $n = 10$; adult vehicle-treated tested on P92, $n = 11$; adult vehicle-treated tested on P106, $n = 13$; adult AAS-treated tested on P92, $n = 12$; adult AAS-treated tested on P106, $n = 10$.

In each experiment, immediately following the behavior test, animals were administered an overdose of sodium pentobarbital (130 mg/kg i.p.). Testes and seminal vesicles were removed and weighed. At the time of sacrifice, flank gland diameters were assessed by shaving the hindquarters and measuring the gland with a caliper.

2.3. Statistical analyses

Three-way ANOVAs were used to determine main effects and interactions between age at treatment (adolescent or adult), time of testing post-treatment (two or four weeks after discontinuation of treatment) and treatment (vehicle or AAS) on aggressive and reproductive behaviors, testes weights, seminal vesicle weights, and flank gland diameters. The three-way ANOVAs revealed no main effect of post-treatment testing time on either sexual or aggressive behaviors. Therefore, data from the two and four week post-treatment test groups were combined, and then two-way ANOVAs were conducted to determine effects of AAS treatment, age of treatment, and interactions on each behavior. Unpaired Student's *t*-tests were used as post hoc planned comparisons to determine significant differences between treatment groups exposed to AAS during adolescence or adulthood. Outliers were identified by Dixon outlier tests and data from these subjects were excluded from statistical analyses.

In Experiment 2, none of the males exposed to AAS during adolescence exhibited any submissive behaviors when tested either two or four weeks post-treatment. Therefore, data from the two and four week post-treatment test groups were combined, and Fisher's Exact Test was used to determine statistical differences in submissive behaviors between males treated with AAS during adolescence and