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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.

Keywords

anabolic steroids; sex behavior; aggression; submissive behavior; adolescence; development

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

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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 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 × 38 × 17 cm) with ad libitum access to food (Teklad 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 1600h EST). At 25 days of age, animals were singly housed in polycarbonate cages (30.5 × 10 × 20 cm, for Experiment 1; 33 × 38 × 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 ml 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.

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 × 26 × 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 μ g in 0.05 ml sesame oil) 48 h before, and a progesterone injection (500 μ g 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 to 30 seconds, 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 minute 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.

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.

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 the other three groups (vehicle treated during adolescence, vehicle treated during adulthood and AAS treated during adulthood). This statistical analysis is used when sample sizes are small, or when the data are very unequally distributed among the cells of a 2×2 contingency table.

Results

Experiment 1: Effects of AAS on sexual behavior

There were no effects of AAS treatment or age of treatment on the amount of time spent in anogenital investigation of the receptive female or on the number of mounts, intromissions, and ejaculations (data not shown). However, three-way ANOVA of long intromissions, an index of sexual satiety in Syrian hamsters [20,26], revealed a significant main effect of treatment. AAS treatment significantly reduced the number of long intromissions [$F(1, 60) = 5.975$; $P < 0.02$] (Figure 1A) and increased the latency to display a long intromission [$F(1, 60) = 5.922$; $P < 0.02$] (Figure 1B). As time of testing post treatment did not affect the expression of long intromissions, the data from the 2 and 4 wk post-treatment test times were combined, a two-way ANOVA was used to determine whether there was an interaction between AAS treatment and age of treatment. This analysis confirmed that the number of long intromissions was significantly less [$F(1, 64) = 6.187$; $P < 0.015$] and the latency to display a long intromission was significantly longer [$F(1, 64) = 6.407$; $P < 0.014$] in AAS-treated males relative to vehicle-treated males, regardless of age of treatment, and revealed no interaction between treatment and age at treatment (Figure 1 insets).

Testes weights, seminal vesicle weights, and flank gland diameters were not different between AAS and vehicle treated males, regardless of age of treatment and when measured either two or four weeks after discontinuation of treatment (Table 1).

Experiment 2: Effects of AAS on agonistic behaviors

Aggressive/Dominant Behaviors—Three-way ANOVA revealed that relative to vehicle controls, AAS treatment resulted in increased number of attacks [$F(1, 84) = 4.579$; $P < 0.05$; Figure 2A] and number of bites [$F(1, 84) = 4.496$; $P < 0.05$; Figure 2B]. After combining data from the two post-treatment test times, two-way ANOVA revealed a main effect of both treatment and age of treatment. Relative to vehicle-treated males, AAS-treated males displayed a greater number of attacks [$F(1, 88) = 5.361$; $P < 0.02$; Figure 2A inset] and bites [$F(1, 88) = 5.151$; $P < 0.03$; Figure 2B inset]. Males treated during adolescence displayed significantly more attacks [$F(1, 88) = 33.019$; $P < 0.001$; Figure 2A inset] and bites [$F(1, 88) = 39.505$; $P < 0.0001$; Figure 2B inset] than males treated in adulthood. However, this statistical effect reflects the younger age of the adolescent-treated males compared with adult-treated males at the time of testing, given the age-related decline in aggressive behavior normally observed in Syrian hamsters [27] and also seen in the vehicle-treated groups in this experiment.

A three-way ANOVA revealed a main effect of treatment [$F(1, 84) = 6.761$; $P < 0.01$] and age of exposure [$F(1, 84) = 18.279$; $P < 0.001$] on attack duration (Figure 3A) and composite aggression score (Figure 3B). After combining data from the two post-treatment test times, two-way ANOVA confirmed the main effects of treatment and age of exposure on attack duration [$F(1, 88) = 7.597$, $P < 0.01$; $F(1, 88) = 20.345$, $P < 0.001$, respectively] and composite aggression score [$F(1, 88) = 32.533$, $P < 0.0001$, $F(1, 88) = 6.725$; $P < 0.001$, respectively]. Relative to vehicle-treated males, AAS-treated males displayed longer attack duration (Figure 3A inset) and had a higher composite aggression score (Figure 3B inset). As with attacks and bites, the statistical effect of age of exposure on attack duration and composite aggression score is most likely related to the normal age-related decline in aggression in Syrian hamsters.

Three-way ANOVA revealed main effects of treatment [$F(1, 84) = 6.13$; $P < 0.02$] and age of treatment [$F(1, 84) = 10.53$; $P < 0.002$] on flank marking behavior (Figure 4). AAS treatment resulted in persistent increases in flank marking behavior over vehicle controls independent of age of AAS exposure, and flank marking was significantly higher in males treated during adolescence, although this latter effect is secondary to differences between adolescent- and adult-treated males in age at testing. A significant interaction was observed between the age of treatment and time of testing [$F(1, 84) = 6.484$, $P < 0.02$], again most likely related to the older age at testing of adult-exposed groups.

Defensive/Submissive Behaviors—Tail-up walking, escape dashes and stretch-attend are defensive or submissive behaviors, and none of the males treated with AAS during adolescence expressed a single instance of any of these behaviors during either of the two post-treatment test times (Table 2). Because post-treatment test time (2 or 4 wks) had no effect on the expression on submissive behaviors, data from these two tests were combined and Fisher's Exact Test was used to determine statistical differences among groups. Males exposed to AAS during adolescence were significantly different from all other groups in all submissive behaviors assessed ($p < 0.001$). These data indicate that AAS result in a long-lasting suppression of submissive behaviors only when AAS exposure occurs during adolescence.

Physiological Measures—There were no effects of treatment on any of the peripheral measures in this experiment, with one exception (Table 1). Two-way ANOVA revealed a main effect of treatment [$F(1, 84) = 22.030$; $P < 0.0001$] and post-treatment test time [$F(1, 84) = 14.003$; $P < 0.0003$] on testes weight. AAS-treated males had smaller testes than vehicle-treated males. The effect of post-treatment test time simply reflects higher testes weights in older animals.

Discussion

These experiments are the first to directly compare the long term consequences of AAS exposure during adolescence and adulthood on male social behaviors. An AAS cocktail, in doses comparable to what a heavy or chronic anabolic steroid human user would administer [28], led to reduced potential for sexual satiety that persisted at least four weeks beyond the period of exposure to AAS, whether subjects were treated in adolescence or in adulthood. Similarly, AAS resulted in persistent increases in aggressive behaviors and flank marking, regardless of the age at which exposure to AAS occurred. In contrast, the long term consequences of AAS on submissive behaviors were more pronounced when exposure occurred during adolescence, as reflected by the complete absence of submissive behaviors in males treated with AAS during adolescence and tested in adulthood. Thus, AAS exposure leads to persistent changes in both sexual and agonistic behaviors in adolescent- and adult-exposed males. Furthermore, the adolescent brain is more susceptible than the adult brain to adverse and long-lasting effects of AAS on some, but not all, male social behaviors.

In experiment one, long-lasting effects of AAS on the performance of sexual behavior *per se* were not observed. This finding stands in contrast to a previous study in rats, in which pubertal AAS exposure resulted in decreases in the expression of sexual behaviors up to 9 wks after AAS withdrawal [13]. A longer period of AAS treatment in the previous study compared with the present study may account for this difference in findings. Nevertheless, AAS treatment in either adolescence or adulthood decreased the expression of long intromissions up to several weeks after AAS withdrawal. Long intromissions are indicative of the onset of sexual satiety [20], which is the inhibition of masculine sexual behavior after repeated ejaculations. A recent study showed that a cocktail of AAS similar to the one administered here increased the number of ejaculations in sexually experienced males 48 hrs after sexual satiety [29]. Satiety is associated with a reduction in androgen receptor [30] and an increase estrogen receptor expression in the forebrain [31]. Androgen receptor expression is up-regulated by chronic administration of synthetic androgens [28]. The anabolic steroids in the cocktail administered in this study were all aromatizable androgens, and can therefore activate either androgen or estrogen receptors both in the brain and periphery. Thus, the long-term effects of AAS on sexual satiety may be mediated by AAS regulation of androgen and estrogen receptor activation and expression.

Dopamine plays a role in sexual motivation and performance [32-36]. In addition, pharmacological manipulations that enhance dopaminergic activity facilitate male sexual behavior [reviewed in 37]. AAS treatment of adult mice and rats increases dopamine synthesis and receptors in the nucleus accumbens [38-42], although a single subcutaneous injection of T does not affect dopamine levels in the nucleus accumbens of Syrian hamsters [43]. In addition, nandrolone decanoate increases D1 receptor mRNA and decreases monoamine oxidase activity in the amygdala [42,44]. Both sexual satiety and testosterone are associated with increased dopamine activity within the medial preoptic area (MPOA) of rats [45], which receives projections from the amygdala [30,35,46]; however it is unknown if dopaminergic tone is affected by this AAS cocktail in the MPOA. Taken together, a hypothesis that could be tested is that AAS exposure exerts long term effects on sexual satiety by dysregulating steroid-sensitive dopaminergic systems by way of increases in dopamine receptors and decreased degradation of catecholamines that mediate rewarding properties of sexual behavior and sexual performance.

The expression of agonistic behavior evolves throughout adolescence and is differentially expressed in juvenile and adult males. Juvenile and adolescent males tend to display more overt aggressive behaviors towards their opponents, while adult males communicate dominance through posturing and an array of both aggressive and submissive behaviors [15,21,47-49]. In the second experiment, we found long term changes in agonistic behaviors (aggressive and submissive behaviors) due to AAS exposure during adolescence and adulthood. First, a significant increase in number of bites, attacks, attack duration and flank marking behavior was observed up to four weeks after discontinuation of AAS treatment, whether treatment occurred in adolescence or in adulthood. Previous studies show that anabolic steroid exposure increases overt offensive aggression in male rodents while activating neural structures involved in mediating agonistic behaviors [15,47,50]. Even though the relationship between synthetic androgens and increased aggression has been well documented in humans and animal models [9,10,51-53], this study is the first to directly compare long-term consequences of adolescent and adult AAS exposure and determine that aggressive behaviors remain heightened long after the discontinuation of drug treatment. Second, it appears that long-term effects of AAS may be more pronounced when exposure occurs during adolescence because submissive behaviors (tail-up walking, escape dashes and stretch-attend) were never observed in males treated during adolescence, in contrast to males exposed to AAS in adulthood. Few studies have reported how AAS affect submissive and risk-assessment behaviors; however we previously demonstrated that AAS acutely suppressed submissive behaviors in adolescent hamsters [15]. To a certain

extent, adolescent AAS-exposed subjects can be compared to pubertal males subjected to social subjugation [27,54] since they exhibit a similar behavioral phenotype of increased aggression and decreased submission as adults. Albeit through different neural pathways [55] these two experimental paradigms show that both pharmacological and experiential manipulations during adolescence have enduring behavioral consequences on the expression of agonistic behaviors in adulthood.

Many studies have focused on the neural circuitry and mechanisms that mediate aggression using the male Syrian hamster as an animal model [21,56-58], and this work has identified serotonin as a key neurotransmitter that modulates this agonistic behavior in both adolescent and adult subjects [22,56,59-61]. A decrease in serotonergic innervations of the amygdala and anterior hypothalamus [62], a decrease in serotonin receptors [63], and a decrease in serotonin release in the hypothalamus [64] have all been observed after adolescent anabolic steroid exposure. The effects of AAS on serotonergic systems are long lasting [63], and may underlie some of the enduring effects on agonistic behaviors observed in this experiment. In addition to changes in serotonergic systems, other neuropeptide and neurotransmitter systems are altered by AAS treatment, for example, glutamate, gamma-aminobutyric acid (GABA) and vasopressin [14,50,65-68]. Although these changes are not persistent, they could lead to other types of enduring alterations in behavioral circuits that also contribute to the behavioral effects we have observed [69-71]. Studies have yet to directly compare how these neural systems are differentially affected by AAS exposure in the adolescent and adult brain.

Recently, we showed that adolescence culminates a developmental period sensitive to the organizational effects of androgens on the expression of reproductive behaviors [72]. These experiments provide evidence for the effects of synthetic androgens on male rodent sexual and agonistic behaviors that persist beyond the period of drug exposure. In addition, our results indicate that the adolescent brain is more vulnerable than the adult brain to the consequences of AAS on agonistic behaviors since adolescent exposure, but not adult exposure, to AAS leads to long-lasting inhibition of submissive behaviors. While long-term effects of AAS on human physiology and health are well documented [73-77], relatively little is known about the potential long-term behavioral consequences of AAS use by adolescent or adult humans. Our findings call for more research on this question, since anabolic steroid use is increasing as the perception of risk of anabolic steroid use is decreasing [52].

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Figure 1. AAS exposure reduces the potential to reach satiety

Three-way (larger graphs) and two-way (smaller insets) ANOVAs determined that AAS exposure during adolescence or adulthood significantly decreases the number of long intrusions (A) and increases long intrusion latency (B) independent of time of behavioral testing and age at treatment. Asterisks in the insets indicate a significant difference ($p < 0.05$) between AAS- and vehicle-treated groups within age of exposure.

Figure 2. AAS treatment results in persistent elevations in the number of attacks and bites

A three-way ANOVA revealed a main effect of treatment and age of exposure on the expression of both (A) attacks and (B) bites. No effects of time of testing or significant interactions were found. A 2-way ANOVA comparing age of treatment and treatment determined that AAS exposure (insets) increased attacks and bites independently of age of treatment. Asterisk and number sign (#) in the insets indicate significant ($p < 0.05$) main effects of treatment and age of treatment, respectively. Main effect of age of treatment reflects the expected higher levels of aggressive behavior of males tested at younger ages (see behavior of males treated with vehicle during adolescence or adulthood).

Figure 3. AAS results in a persistent increase in attack duration and overall aggression

Three-way ANOVA revealed a main effect of treatment and age of exposure on (A) attack duration and (B) the composite aggression score. No effects of time of testing or significant interactions were found. Two-way ANOVA comparing age of exposure and treatment determined that AAS exposure increased attack duration and overall aggression (insets). Asterisk and number sign (#) in the insets indicate significant ($p < 0.05$) main effects of treatment and age of treatment, respectively. Main effect of age of treatment reflects the expected higher levels of aggressive behavior of males tested at younger ages (see behavior of males treated with vehicle during adolescence or in adulthood).

Figure 4. AAS results in persistent increases in flank marking behavior

Three-way ANOVA showed that flank marking behavior was significantly increased by AAS treatment and revealed an interaction between age of exposure and time of testing post treatment. The interaction reflects age-related changes in flank marking behavior, which can be seen in the vehicle-treated groups..

Table 1
Physiological measures
Peripheral measures in males exposed to AAS during adolescence or adulthood.

	Adolescent AAS Treatment				Adult AAS Treatment			
	Vehicle treated (Mean +/- SEM)		AAS-treated (Mean +/- SEM)		Vehicle Treated (Mean +/- SEM)		AAS-treated (Mean +/- SEM)	
Age of testing	P56	P70	P56	P70	P92	P106	P92	P106
Testing post-treatment	2 wks	4 wks	2 wks	4 wks	2 wks	4 wks	2 wks	4 wks
Testes Weight (g)								
Experiment 1	3.43 ± 0.1	3.41 ± 0.7	3.11 ± 0.3	3.81 ± 0.6	3.93 ± 0.4	4.04 ± 0.3	3.75 ± 0.5	3.77 ± 0.8
Experiment 2 *	3.10 ± 0.2	3.53 ± 0.2	2.87 ± 0.3	3.39 ± 0.3	3.87 ± 0.2	3.75 ± 0.2	3.21 ± 0.6	3.45 ± 0.4
Seminal Vesicle Weight (g)								
Experiment 1	0.19 ± 0.07	0.22 ± 0.04	0.26 ± 0.03	0.24 ± 0.05	0.31 ± 0.04	0.33 ± 0.06	0.30 ± 0.05	0.35 ± 0.07
Experiment 2	0.21 ± 0.07	0.27 ± 0.04	0.24 ± 0.06	0.25 ± 0.06	0.34 ± 0.10	0.35 ± 0.06	0.37 ± 0.08	0.37 ± 0.10
Flank Gland Diameter (mm)								
Experiment 1	8.48 ± 0.6	8.35 ± 1.2	7.77 ± 2.1	8.59 ± 1.6	8.88 ± 0.9	8.63 ± 0.8	9.48 ± 1.6	8.74 ± 0.6
Experiment 2	7.04 ± 1.11	7.87 ± 0.62	7.02 ± 1.32	7.58 ± 0.60	8.73 ± 1.07	7.54 ± 1.07	8.70 ± 1.09	8.30 ± 0.80

* A main effect of treatment was found on testes weight in Experiment 2 (p < 0.001).

Table 2**Submissive behaviors**

Males treated with AAS during adolescence and tested two or four weeks after discontinuation of treatment did not display submissive behaviors. Numbers indicate the proportion of subjects in each group that displayed at least one instance of tail-up walking, escape dashes or stretch-attend behaviors.

Submissive Behavior	Adolescent exposure – Vehicle treated (n=22)	Adolescent exposure – AAS treated (n=24)	Adult exposure – Vehicle treated (n=24)	Adult exposure – AAS treated (n=22)
Tail-Up Walking	8/22 *	0/24	14/22 **	13/22 **
Escape Dashes	8/22 *	0/24	14/22 **	14/22 **
Stretch-attend	6/22 *	0/24	17/22 **	13/22 **

* $p < 0.001$

** $p < 0.0001$ in Fisher's Exact Test analysis of frequency of behavior in AAS-treated during adolescence vs. each of the other three groups.