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Effects of anabolic androgenic steroids and social subjugation on behavior and neurochemistry in male rats

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ABSTRACT

Early abuse and anabolic androgenic steroids (AAS) both increase aggression. We assessed the behavioral and neurochemical consequences of AAS, alone or in combination with social subjugation (SS), an animal model of child abuse. On P26, gonadally intact male rats began SS consisting of daily pairings with an adult male for 2 weeks followed by daily injections of the AAS, testosterone on P40. As adults, males were tested for sexual and aggressive behaviors towards females in various hormonal conditions and inter-male aggression in a neutral setting using home or opponent bedding. Neurotransmitter levels were assessed using HPLC. Results showed that AAS males displayed significantly more mounts toward sexually receptive, vaginally obstructed females (OBS) and displayed significantly more threats towards ovariectomized females. SS males mounted OBS females significantly less and were not aggressive toward females. The role of olfactory cues in a neutral setting did not affect aggression regardless of treatment. AAS significantly increased brainstem DOPAC and NE. SS decreased 5HIAA, DA, DOPAC, and NE in brainstem. 5HIAA was significantly increased in the prefrontal cortex of all experimental groups. We conclude that AAS and SS differentially affect behavior towards females as well as neurotransmitter levels.

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1. Introduction

Anabolic androgenic steroid (AAS) use among teenage males is a major health concern because androgenic influences play a major role in brain development as well as the expression of adaptive adult social behaviors (Primus and Kellogg, 1990; Romeo, 2003; Sisk and Foster, 2004; Sisk et al., 2003; Spear, 2000). In animal models exposure to AAS during adolescence alters both brain neurochemistry and adult behavior patterns (Farrell and McGinnis, 2003; Keleta et al., 2007; Kubala et al., 2008; McGinnis et al., 2002; Wesson and McGinnis, 2006). AAS increases aggression in mice (Martinez-Sanchis et al., 1998), hamsters (Harrison et al., 2000; Melloni et al., 1997), and rats (Breuer et al., 2001; Farrell and McGinnis, 2003; Feinberg et al., 1997; Lumia et al., 1994; McGinnis et al., 2002; Wesson and McGinnis, 2006) as well as humans (Choi and Pope, 1994; Galligani et al., 1996; Perry et al., 2003; Pope et al., 2000). Increased aggression towards women by male AAS users has been reported (Choi and Pope, 1994). In agreement with the human data, increased aggression toward females has been confirmed in a rat model of AAS use (Cunningham and McGinnis, 2006, 2007). Interestingly, the AAS-induced aggression was only displayed toward non-receptive (ovariectomized) females. When females were sexually receptive, aggression was absent and copulatory behavior was displayed.

An interesting parallel arises between adolescent AAS users and abused children, as both display increased aggression toward males (Connor et al., 2003; Lansford et al., 2002) and females (Wekerle et al., 2001; 2009; Wolfe et al., 2001). Social subjugation (SS) has been used as an animal model to assess the impact of early abuse on the development and expression of adaptive social response patterns. During SS, an animal is dominated by a larger conspecific male in several encounters (Cunningham and McGinnis, 2008; Delville et al., 1998; Ferris et al., 2005; Wommack and Delville, 2003; Wommack et al., 2004). As often observed in abused children, male rats exposed to SS prepubertally display increased aggression in adulthood (Cunningham and McGinnis, 2008). Also, males who have been abused in childhood are more likely to take AAS in adolescence (Skarberg and Engstrom, 2007).

In rodent studies, exposure to AAS during adolescence has been found to alter serotonin, but both increases and decreases have been reported depending on the brain region. For example, AAS decreases 5-HT and its metabolite 5-HIAA in the hypothalamus (Keleta et al., 2007; Kubala et al., 2008), medial amygdala (Grimes and Melloni, 2006), striatum (Keleta et al., 2007) and hippocampus (Bonson et al., 1994). However, in the frontal cortex, AAS has been consistently found to increase serotonin (Keleta et al., 2007; Kubala et al., 2008; Kurling et al., 2005). These data support a role for serotonin in modulating the effects of AAS.

AAS have also been found to increase dopamine (DA) and its metabolite, DOPAC, in the anterior hypothalamus (Ricci et al., 2009) the striatum (Kindlundh et al., 2002, 2004) and the cortex (Thiblin

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et al., 1999; Kurling et al., 2005). The effect of AAS on NE is currently unknown.

The effect of SS on brain neurochemistry has not been previously investigated. Since AAS users may have a childhood background of abuse (Skarberg and Engstrom, 2007), the impact of combining SS and AAS on serotonin and dopamine levels may provide insight into the long-lasting effects of periadolescent experiences on brain neurochemistry.

The overall goal of this study was to determine the influence of prepubertal SS, and adolescent AAS, alone or in combination, on behavior and brain neurochemistry. Since both AAS and SS increase aggression toward males, we tested the hypothesis that aggressive responses and changes in neurochemistry would be comparable. First, we examined whether prepubertal SS would increase aggression toward females as previously shown following adolescent AAS exposure. Second, we determined whether olfactory cues would be a sufficient to evoke inter-male aggression in SS and AAS males. Third, we measured serotonin, dopamine and norepinephrine following AAS and SS exposure to determine if neurochemical changes were similar to those previously found in AAS-treated males. The results indicated that SS and AAS probably increase inter-male aggression via different underlying mechanisms.

2. Materials and methods

2.1. Animals

All animals were Long-Evans rats obtained from Charles River Laboratory (Wilmington, MA). Animals in experimental groups were 25 days of age upon arrival. A separate group of larger (at least 350 g) adult males were used as subjugators. An additional group of adult males in the same age and weight range as the experimental animals served as opponents in aggression tests. All males were gonadally intact. Females used as stimulus animals were ovariectomized as previously described (Farrell and McGinnis, 2004). The animals were housed in a temperature-controlled room (23 °C) in standard Plexiglas cages (25×20×18 cm) with their bedding changed twice a week. Experimental and opponent males were individually housed, while stimulus females were housed two per cage. Food and water were provided ad libitum. Lights were maintained on a 12:12 reversed light/dark cycle with lights off at 1200 h. Body weights were taken weekly. All drugs used in this study were obtained from Sigma, St. Louis, MO, USA. Experimental procedures were performed in accordance with the National Institute of Health's guidelines for animal care and use. Experimental protocols were reviewed and approved by the UTHSCSA Institutional Animal Care and Use Committee.

2.2. Experimental design

Experimental males were matched for body weight and randomly assigned to one of four treatment groups: social subjugation (SS; $n=8$); testosterone (AAS; $n=8$); social subjugation plus testosterone (AAS+SS; $n=8$), or control ($n=8$). Social subjugation (or control condition) was initiated on P26 and continued until puberty (P40). AAS (or vehicle control) treatment began on P40 and continued 5 days/week until the end of the experiment. Weekly behavioral tests began on P69 (see Fig. 1). These tests consisted of four sexual behavior and aggression tests with females in different hormonal conditions and two tests for inter-male aggression in a neutral cage with home or opponent male bedding in different conditions (with and without physical provocation). All behavioral tests were conducted during the dark phase of the light cycle under dim red light. On P92 experimental animals were sacrificed by decapitation for HPLC analysis.

2.3. Social subjugation

The animals underwent social subjugation during the dark phase of the light cycle for 10 min/day, 5 days/week from P26 to P40. SS and AAS+SS animals were individually placed into the home cage of a subjugator, a larger adult male. Controls and males receiving AAS alone were placed into a novel cage with fresh bedding (Bastida et al., 2009; Cunningham and McGinnis, 2008; Delville et al., 1998; Ferris et al., 2005; Wommack and Delville, 2003). Each test of social subjugation was observed to ensure that the subjugator exhibited acts of aggression such as dominance postures and mounts (Cunningham and McGinnis, 2008). The subjugator males typically do not injure the experimental animals.

2.4. AAS exposure

AAS exposure was initiated on P40, the time of preputial separation (Korenbrot et al., 1977), which is used as an indicator for the onset of puberty. AAS and AAS+SS males began receiving sc injections of testosterone propionate at a dosage of 5 mg/kg dissolved in polyethylene glycol 200 (PEG-200) while control and SS males received vehicle injections (Cunningham and McGinnis, 2006; Farrell and McGinnis, 2003, 2004; McGinnis et al., 2002; Wesson and McGinnis, 2006).

2.5. Social interactions with females

Males were paired with females under four different conditions. All four groups were tested on the same day for each condition. Males were tested for both sexual and aggressive behaviors with females in a 25×20×18 cm glass chamber over a 10 min test period (Cunningham and McGinnis, 2006). In the first test each male was paired with an ovariectomized, non-receptive female (OVX). For the second test each male was paired with a different sexually receptive female that had received sc implantation of one silastic capsule (1.47 mm i.d.× 1.96 mm o.d.×5 mm length, Dow Corning, Midland, MI) containing 50% crystalline estradiol benzoate followed on the day of testing with a sc injection of 500 μg progesterone 4 h prior to the start of the test (E+P). The third and fourth tests were designed to examine the males' response to females under conditions that would elicit a state of frustration. For the third test, experimental animals were tested with E+P females whose vaginas were obstructed with duct tape to prevent intromissions (OBS group) (Cunningham and McGinnis, 2007). Finally, in the fourth test the males were tested with OVX females while being physically provoked (OVX+Prov). Physical provocation was administered by tail pinch with forceps to the distal end of the experimental animal's tail once every minute for the

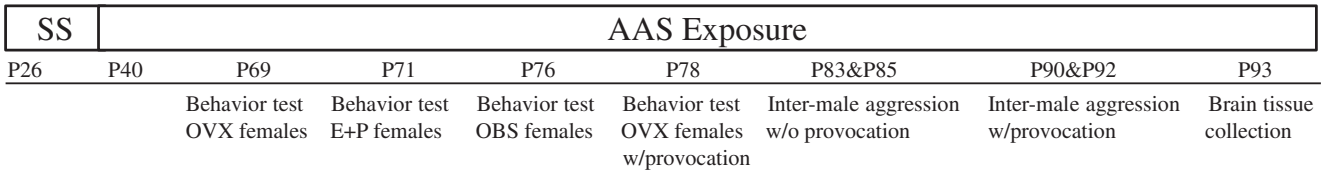

Fig. 1. Experimental timeline schematically depicting the schedule of social subjugation (SS) and anabolic androgenic steroids (AAS) injections, behavioral testing, and brain tissue collection. P = postnatal day.

duration of the 10 min test (Cunningham and McGinnis, 2006; Farrell and McGinnis, 2004; Smith et al., 1997).

For all tests with females, both sexual and aggressive behaviors were recorded. Sexual behaviors scored included mount and intromission frequencies, mount and intromission latencies, and ejaculations (Cunningham and McGinnis, 2007; Farrell and McGinnis, 2004; Wesson and McGinnis, 2006). Aggressive behaviors towards females consisted of attacks, threats, boxing, and mounts that were not associated with intromissions or ejaculations (Barfield et al., 1972; Cunningham and McGinnis, 2007).

2.6. Aggression tests with males

All males were tested using a modified resident-intruder paradigm to assess the role of olfactory cues in a neutral setting in facilitating aggression. Experimental and opponent males were individually housed and their bedding was not changed for seven to ten days prior to testing to facilitate home cage olfactory recognition (Barfield et al., 1972; Breuer et al., 2001; Wesson and McGinnis, 2006). The aggression tests were conducted in a neutral glass chamber (25×20×18 cm) that neither the experimental male nor the intruder male had ever occupied. To create the olfactory milieu of the resident or intruder male, the soiled bedding of either the experimental or opponent animal was placed on the floor of the neutral test cage. The animals were tested twice without provocation, once with their home bedding and once with the opponent's home bedding. The order of bedding presentation was counterbalanced to control for order effects that might influence the level of aggression. The following week the animals were tested for aggression with physical provocation, also with the bedding of the experiment animal and the home bedding, and the bedding conditions counterbalanced. Physical provocation was administered to the experimental animal as described earlier (Cunningham and McGinnis, 2006; McGinnis et al., 2002). Aggression tests were videotaped for subsequent analysis (Breuer et al., 2001). Behaviors were scored only when they were initiated by the experimental male (Cunningham and McGinnis, 2007). Aggressive behaviors included mounts, threats, dominant postures, and attacks (Farrell and McGinnis, 2004). These behaviors were combined to derive a composite aggression score (Cunningham and McGinnis, 2007; Farrell and McGinnis, 2004; Nomura et al., 2002; Wesson and McGinnis, 2006).

2.7. Determination of DA, 5-HT, and metabolites

On P92 experimental animals were sacrificed by decapitation. Brains were quickly removed and placed in a freezing chamber until dissected. The brain areas selected for HPLC analysis were the striatum, cortex, hippocampus, hypothalamus, and brain stem and dissected as described previously (Keleta et al., 2007). The striatum included the caudate putamen from the beginning of the corpus callosum to the optic chiasm. For the cortex, 2 mm of the frontal pole anterior to the beginning of the corpus callosum. The hypothalamus included from the optic chiasm to just posterior to the mammillary bodies. The brainstem contained the entire brainstem anterior to the superior colliculus to 0.5 mm posterior to the inferior colliculus. These brain regions were selected to determine serotonin function in areas specific to gonadal steroid mechanisms related to androgen-dependent behaviors (hypothalamus, hippocampus and frontal cortex) and in the cell body (brainstem) region (Keleta et al., 2007). All tissue samples were placed into plastic vials on dry ice then stored at −80 °C until processed and analyzed. For HPLC, frozen brain tissue samples were homogenized in ice-cold 0.1 M perchloric Acid ($HCLO_4$) containing 10 ng/mL DHBA, an internal standard. Samples were placed on ice for two minutes, and then centrifuged at 15,294×*g* for 2 min (Eppendorf 5810 centrifuge). An aliquot of the supernatant was filtered through a 0.45-mm Millipore Cor. (Billerica, MA) microcentrifuge filter and then centrifuged at 12,000 rpm for 1 min. Norepinephrine, 5-HT, 5HIAA, DA, and DOPAC were separated using a high-performance liquid chromatography (HPLC) system that consisted of a dual-piston pump (Solvent Delivery Module-Model 580), a refrigerated autosampler (Model 540), and a Coulochem II (Model 500; all ESA Biosciences Inc., Chelmsford, MA) dual-potentiostat electrochemical detector. Data collection and system control were performed using a PC-based data station (Model 500). Separation of catecholamines and metabolites was achieved on an HR-80 reverse-phase C18 column (4.6×80 mm). Analytes were detected on a dual-electrode analytical cell (Model 5011A) with the first electrode (E1) set at −50 mV and the second electrode (E2) set to oxidize catecholamines and its metabolites at +280 mV. A guard cell (Model 5020) was placed between the pump and the autosampler at a potential of +350 mV to oxidize contaminants in the mobile phase. The mobile phase consisted of 75 mM sodium phosphate monobasic, 4.0 mM heptanesulfonic acid, 25 μM EDTA, 0.01% triethylamine, and 6% acetonitrile (v/v). The pH of the mobile phase was adjusted to 3.1 with phosphoric acid after the addition of organic modifiers. The mobile phase was passed through the system at 1.0 mL/min. All analyses were performed at 27 °C and in triplicates. $N=8$ for all brain regions in each group except in the brainstem for NE ($n=4$).

2.8. Statistical analyses

All data were analyzed using StatView v5.0 (Abacus Concepts Inc., Berkeley California). Separate one-way ANOVA's were conducted for each test condition to identify differences between the four treatment groups. This was followed by Fisher's PLSD for post-hoc comparisons for each behavior test and for each brain region and neurotransmitter assessed. Values of $p<0.05$ were viewed as significant.

3. Results

Fig. 2 shows the mean mount frequencies in pairings with E+P, OBS, OVX or OVX+Prov females. AAS males mounted significantly more when paired with sexually receptive females with obstructed vaginas (OBS) as compared to controls ($F(3,28)=2.81$, $p<0.05$). SS males mounted significantly less than controls when paired with the OBS females ($F(3,28)=2.81$, $p<0.05$).

Fig. 2 also depicts the mean mount latencies for males in each of the four treatment groups. There was no significant effect of either AAS or SS on mount latency when the animals were tested with receptive females whether the vaginas were obstructed or not (E+P and OBS). In the absence of provocation, none of the males mounted the OVX females. When provoked, AAS males displayed a significant decrease in mount latency toward OVX+Prov females compared to control ($F(3,28)=5.13$, $p<0.02$) and SS males $F(3,28)=5.13$, $p<0.002$). The combination of AAS+SS significantly decreased mount latency when compared to SS alone ($F(3,28)=5.13$, $p<0.005$), but did not quite reach statistical significance when compared to controls ($p<0.06$).

Males displayed intromissions and ejaculations only when paired with the sexually receptive (E+P) females and there were no significant differences between groups (data not shown). There was no significant difference between groups in any of the tests with EB+OBS, OVX or OVX+Prov females for intromission frequencies, intromission latencies, or ejaculations as intromissions and ejaculations were not exhibited.

There were no significant differences in aggression toward females for boxing or attacks, but there was an effect on threats. Fig. 3 shows the mean number of threats exhibited towards E+P, OBS, OVX and OVX+Prov females. There was a significant increase in the number of threats by AAS males towards OVX females as compared to all other groups (AAS vs control: $F(3,28)=4.58$, $p<0.003$); AAS vs SS ($F(3,28)=4.58$, $p<0.003$); and AAS vs AAS+SS ($F(3,28)=4.58$, $p<0.02$),. There were no significant differences between any other groups during the

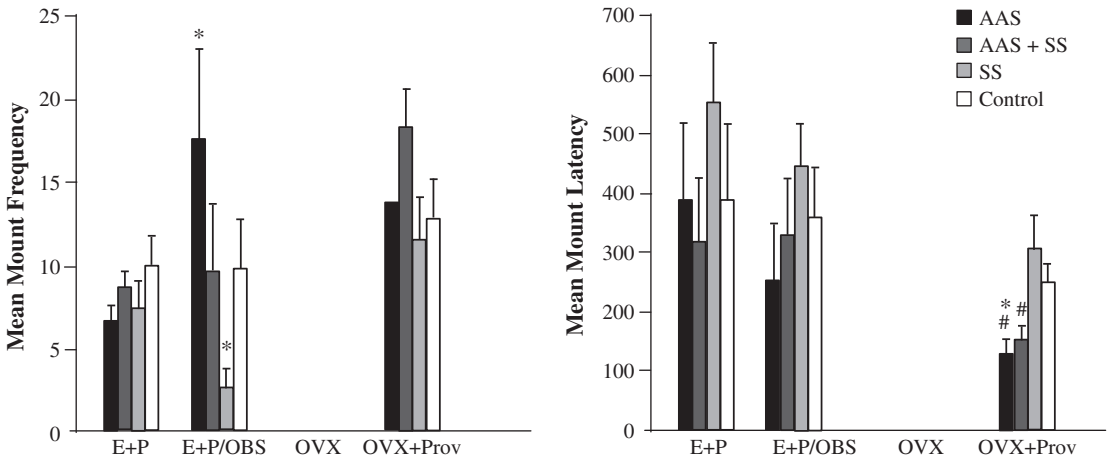

Fig. 2. Mean mount frequencies (mean ± SEM) and mean mount latencies (mean ± SEM) in 10 min tests with females. Socially subjugated males (SS), anabolic androgenic steroid males (AAS), AAS + SS males, and control males were tested with sexually receptive females (E + P), vagina obstructed sexually receptive females (OBS), ovariectomized females (OVX), and ovariectomized females with physical provocation of the experimental animal (OVX + Prov). * = significantly different from controls ($p<.05$ for mount frequency, and $p<.02$ for mount latency); # = significantly different from SS group (AAS vs SS: $p<.002$, and AAS + SS vs SS: $p<.005$). $n=8$ for all groups.

E + P, OBS, or OVX + Prov as no threats were exhibited during these tests.

For inter-male aggression in a neutral cage, there were no significant differences between groups in individual aggressive behaviors or composite aggression scores in the presence of either the experimental or opponent home bedding (Table 1). However, there was an overall significant increase ($p<0.001$) in aggression following provocation compared to the no provocation condition indicating that provocation induces aggression in a previously non-threatening situation for all groups.

Fig. 4 illustrates the levels of 5-HT, 5HIAA, DA, DOPAC and NE in the brainstem, prefrontal cortex, and striatum for each of the treatment groups. In the prefrontal cortex, a significant increase in 5HIAA was found for all groups compared to controls: AAS vs control ($F(3,27)=3.25$, $p<0.03$), SS vs control ($F(3,27)=3.25$, $p<0.008$), and AAS + SS vs control ($F(3,27)=3.25$, $p<0.02$). There were no significant differences between groups in the prefrontal cortex for 5-HT, DA, DOPAC or NE. There were no significant differences in 5-HT, 5-HIAA, DA, or DOPAC in the striatum. However, there was a significant decrease in striatal NE for SS males compared to controls ($F(3,25)=2.95$, $p<0.03$). For the brainstem, AAS treatment resulted in a significant increase in DOPAC ($F(3,25)=5.85$, $p<0.04$), and NE ($F(3,12)=4.17$, $p<0.02$) compared to controls. The combination of AAS + SS significantly increased DOPAC compared to controls ($F(3,25)=5.85$, $p<0.05$). Social subjugation (SS) significantly decreased the levels of all neurotransmitters measured in brainstem: 5-HT ($F(3,16)=3.11$, $p<0.05$); 5-HIAA ($F(3,27)=4.30$, $p<0.02$); DA ($F(3,26)=3.44$, $p<0.02$); DOPAC ($F(3,25)=5.85$, $p<0.01$), and NE ($F(3,25)=2.95$, $p<0.03$) compared to controls. No significant differences were found in the hippocampus or hypothalamus for any treatment group (data not shown).

Fig. 5 shows the mean body weights for each of the four treatment groups (mean ± S.E.M.). Compared to controls, AAS, SS, and AAS + SS all had significantly lower body weights on P54, P61, P68 and P75 ($p<0.05$), and for AAS and SS on P82 ($p<0.05$).

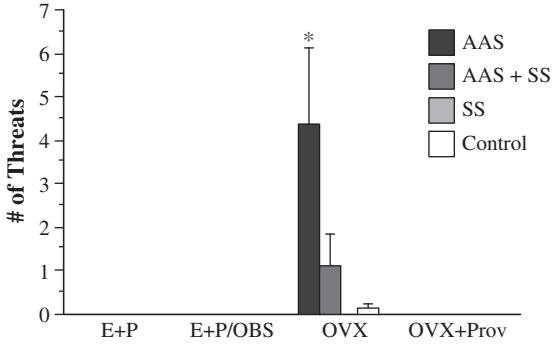

Fig. 3. Mean number of threats (mean ± SEM) in tests with females. Socially subjugated males (SS), anabolic androgenic steroid males (AAS), AAS + SS males, and control males were tested with sexually receptive females (E + P), vagina obstructed sexually receptive females (OBS), ovariectomized females (OVX), and ovariectomized females with physical provocation of the experimental animal (OVX + Prov). * = significant increase compared to controls ($p<0.05$). $n=8$ for all groups.

4. Discussion

This study examined the impact of prepubertal SS and adolescent AAS exposure on aggression toward females. Both human and animal studies have found that AAS exposure increases aggression toward females (Choi and Pope, 1994; Cunningham and McGinnis, 2006, 2007). Animal studies have reported that AAS exposure increases aggression toward females, but this is mediated in part by the hormonal status of the female, frustration (thwarting the opportunity to achieve ejaculation), and physical provocation. We found that AAS males displayed sexual behavior and no aggression toward sexually receptive females supporting previous findings (Cunningham and McGinnis, 2006, 2007). AAS also induced a significant increase in sexual mounting toward receptive females with vaginal obstruction (OBS), which is also consistent with prior results (Cunningham and McGinnis, 2007). Frustration has been defined as an emotional state

Table 1
Inter-male aggression.

	No provocation		Provocation	
	Home bedding ($n=8$)	Opponent bedding ($n=8$)	Home bedding ($n=8$)	Opponent bedding ($n=8$)
AAS	3.75±1.60	2.38±1.16	6.75±2.12	3.25±1.25
AAS + SS	3.13±1.14	2.75±1.41	7.13±3.55	5.50±1.97
SS	3.25±1.29	3.13±3.57	10.4±3.56	5.25±1.93
Control	4.00±1.74	3.88±2.42	11.5±1.76	10.0±2.33

Neutral setting with home or opponent bedding on inter-male aggression. Composite aggression scores are expressed as means ± SEM for socially subjugated males (SS), anabolic androgenic steroid males (AAS), AAS + SS males, and control males. There was an overall significant increase ($p<0.001$) in aggression following provocation compared to no provocation.

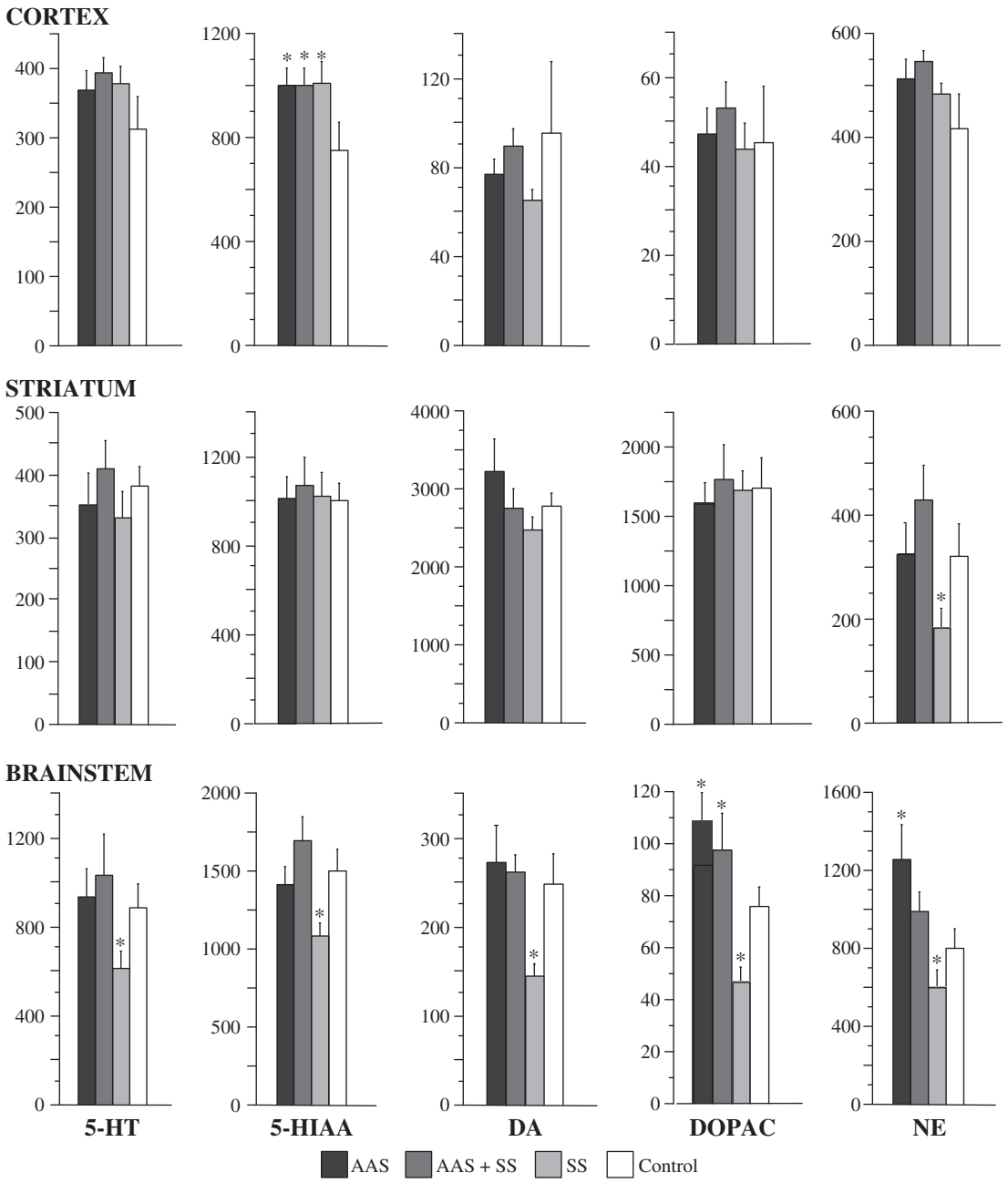

Fig. 4. Brain regional content of NE, DA, DOPAC, 5-HT, and 5HIAA levels (mean ± SEM) for socially subjugated males (SS), anabolic androgenic steroid males (AAS), AAS + SS males, and control males. Data are expressed as ng/mg protein. * = significant difference relative to controls. Individual *p* values can be found in the text.

or condition that results when a goal is thwarted or blocked, preventing gratification (Amsel, 1990). Accordingly, the inability to achieve the goal, in this case ejaculation, should induce frustration-induced aggression. However, in some situations, the inability to fulfill the goal or consummate an act induces a state of persistence (Amsel and Roussel, 1952; Azrin et al., 1966; Cunningham and McGinnis, 2007; Harrell, 1973; Matzel, 1984). These data support the view that when females emit hormonal and behavioral cues indicating sexual receptivity, (proceptivity and attraction) withholding the reward induces persistence, rather than aggression (Beach, 1976). AAS-treated males did not display either intromissions or ejaculations toward non-receptive (OVX) females and, in fact, displayed aggression. AAS males exhibited significantly more threats toward non-receptive females than control males, which are consistent with previous reports (Cunningham and McGinnis, 2006, 2007). These results strengthen the finding that AAS increases aggression towards females that are either not sexually receptive or are prevented from performing normal sexual behavior.

Males abused in childhood are reported to be aggressive toward women (Wekerle et al., 2001, 2009; Wolfe et al., 2001). Contrary, to this report in humans, we found that SS males were not aggressive in any encounter with females and, in fact, when placed with non-receptive females, SS males were the only group that did not display threats. Furthermore, males receiving the combination of AAS + SS failed to display aggressive behaviors towards females. This is the first study to examine social subjugation, alone or in combination with AAS, on aggression towards females in different hormonal conditions. It appears that early social subjugation does not increase aggression toward females.

In male hamsters, SS during adolescence decreased the latency to mount sexually receptive females (Ferris et al., 2005). We found no effect of SS on sexual behavior toward sexually receptive females.

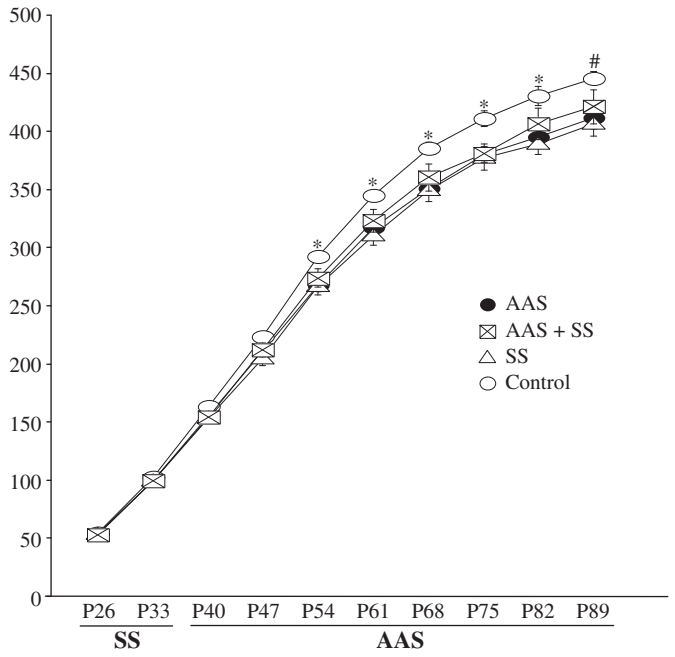

Fig. 5. Weekly body weight measurements (mean ± SEM) for socially subjugated males (SS), anabolic androgenic steroid males (AAS), AAS + SS males, and control males. Beginning at P54, all experimental groups had a significant decrease in body weight as compared to controls ($p<0.05$). On P82, AAS and SS males had a significant decrease in body weight as compared to controls ($p<0.05$). $n=8$ for all groups.

There was, however, a significant decrease in mount rate toward non-receptive females as well as an increase in mount latency when tested with OVX females in the provocation condition. Sexual behavior towards females was not affected by the combination of AAS + SS. Taken together, these data suggest that early social subjugation may decrease the likelihood to copulate under certain conditions.

The role of olfactory cues in the elicitation of aggression in SS and AAS male rats was examined by measuring aggression in a neutral cage in the presence of the experimental or opponent home bedding. We predicted that both SS and AAS males would display elevated levels of aggression in the presence of their home bedding and in the presence of the opponent's home bedding. This is based on prior studies in both pubertal and adult AAS-treat rats (Breuer et al., 2001; Farrell and McGinnis, 2003; McGinnis et al., 2002) showing that AAS-treated males displayed enhanced aggression when tested in their home cage and in their opponent's home cage, but not in a neutral cage. AAS males tested in a neutral cage with clean bedding exhibited levels of aggression that were similar to gonadally intact vehicle control males (Breuer et al., 2001). The current study shows that olfactory cues are not sufficient to elevate aggression following either AAS or SS alone or in combination. This was demonstrated by the finding that neither the presence of experimental nor opponent animal's home cage bedding elicited aggression when the encounter occurred in a neutral cage. Therefore, the home cage itself may be a critical factor for eliciting aggression.

It has previously been demonstrated that both AAS and SS exposures increase inter-male aggression (Breuer et al., 2001; Cunningham and McGinnis, 2008; Delville et al., 1998; Farrell and McGinnis, 2003; Feinberg et al., 1997; Ferris et al., 2005; Harrison et al., 2000; Lumia et al., 1994; Martinez-Sanchis et al., 1998; Melloni et al., 1997; McGinnis et al., 2002; Wesson and McGinnis, 2006). Since aggression is thought to be mediated in part by serotonin, we hypothesized that AAS and SS would have similar effects on serotonin levels. AAS significantly reduces 5-HT levels in the striatum of rats (Keleta et al., 2007), medial amygdala in hamsters (Grimes and Melloni, 2006) and hippocampus of mice (Bonson et al., 1994). However, in the current study, AAS had no effect on 5-HT. The differences in results may be attributed to variations in experimental methodologies, different HPLC procedures and species differences. We found a significant increase in 5-HIAA in the prefrontal cortex in all groups (AAS, SS and AAS + SS) compared to controls, suggesting increased turnover in 5-HT. This is generally consistent with previous reports of an increase in 5-HT in the prefrontal cortex (Keleta et al., 2007; Kubala et al., 2008) and suggest a potential role of 5-HT in the prefrontal cortex in mediating the effects of AAS on a host of affective behaviors. In contrast to AAS, we found that SS significantly decreased brainstem 5-HT levels. In a study on hamsters, SS increased 5-HT varicosities in the anterior hypothalamus (Delville et al., 1998), but we found no effect of SS on 5-HT in the hypothalamus. No other effects of AAS or SS on 5-HT levels were found.

Although our primary focus was on serotonin, by using HPLC we were also able to assess DA, DOPAC and NE levels in the brain. We found that AAS significantly increased DOPAC and NE in the brainstem. This is consistent with previous reports that AAS increases DA function (Kindlundh et al., 2002; Kindlundh et al., 2004; Ricci et al., 2009). Taken together, these data suggest a possible role for altered dopaminergic activity in modulating the behavioral effects of AAS. In contrast to AAS, social subjugation resulted in a ubiquitous decrease in brainstem DA, DOPAC and NE. Overall, our results suggest that the early experience of social subjugation may have long-lasting repercussions on brain neurochemistry. Since these measures were taken in adulthood, this suggests that early hormonal or social challenges may reorganize neurotransmitter function selectively and thereby induce changes in adult social behavior patterns.

AAS, SS, and AAS + SS males weighed significantly less than control males beginning at week 5, which is consistent with previous findings (Cunningham and McGinnis, 2008; Farrell and McGinnis, 2003; Feinberg et al., 1997; Gentry and Wade, 1976; Lumia et al., 1994; Wesson and McGinnis, 2006). The effect of SS on body weight was varied. SS reportedly increases body weight in hamsters (Delville et al., 1998) but not rats (Cunningham and McGinnis, 2006). In the current study, body weights of SS rats were significantly lower than controls four weeks after SS was initiated and two weeks after AAS administration began. This needs further investigation.

Based on our finding that both AAS and SS increase aggression toward males, we hypothesized that SS and AAS males would display aggression towards females. The AAS-induced increase in aggression towards females was consistent with our previous finding (Cunningham and McGinnis, 2006). However, social subjugation had a clear inhibitory effect on both sexual and aggressive behaviors toward females. This was unexpected and indicates that there are marked differences in the behavioral effects of the early experience of SS and adolescent AAS exposure. We originally hypothesized that prepubertal SS would escalate aggression induced by adolescent exposure to AAS, and it does not. In fact, there may be a tendency for AAS to counter the effects of SS. This may reflect the powerful influence of androgens on behavior, rather than a specific interaction with preexisting experiences. Given the importance of olfactory cues in mediating social behaviors in rats, we hypothesized that both AAS and SS would increase inter-male aggression in a neutral cage with home bedding. However, our results show that olfactory cues from either the animal's home bedding or the opponent's home bedding are not sufficient to induce aggression. Thus, the home cage itself plays an essential role in the modulation of AAS and SS induced aggression. Finally, we hypothesized that the changes in neurochemistry between AAS and SS males would be similar. In fact, they had little in common. Social subjugation and AAS both increased cortical serotonin levels, but only SS resulted in a significant decrease in all neurotransmitter measures in the brainstem. Overall, our results indicate that both adolescent AAS exposure and the early experience of social subjugation selectively alter brain neurochemistry and this influences behavior in adulthood. However, the substantial differences in behavior and brain

neurochemistry suggest that the underlying neural mechanisms may be different.

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References

Amsel A. Arousal, suppression, and persistence: frustration theory, attention, and its disorders. Cogn Emot 1990;4:239–68.
Amsel A, Roussel J. Motivational properties of frustration. I. Effect on a running response of the addition of frustration to the motivational complex. J Exp Psychol 1952;43: 363–6.
Azrin NH, Hutchinson RR, Hake DF. Extinction-induced aggression. J Exp Anal Behav 1966;9:191–204.
Barfield RJ, Busch DE, Wallen K. Gonadal influence on agonistic behavior in the male domestic rat. Horm Behav 1972;3:247–59.
Bastida CC, Puga F, Delville Y. Risk assessment and avoidance in juvenile golden hamsters exposed to repeated stress. Horm Behav 2009;55:158–62.
Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. Horm Behav 1976;7:105–38.
Bonson KR, Johnson RG, Fiorella D, Rabin RA, Winter JC. Serotonergic control of androgen-induced dominance. Pharmacol Biochem Behav 1994;49:313–22.
Breuer ME, McGinnis MY, Lumia AR, Possidente BP. Aggression in male rats receiving anabolic androgenic steroids: effects of social and environmental provocation. Horm Behav 2001;40:409–18.
Choi PY, Pope HG. Violence toward women and illicit androgenic-anabolic steroid use. Ann Clin Psychiatry 1994;6:21–5.
Connor DF, Doerfler LA, Volungis AM, Steingard RJ, Melloni RH. Aggressive behavior in abused children. Ann NY Acad Sci 2003;1008:79–90.
Cunningham RL, McGinnis MY. Physical provocation of pubertal anabolic androgenic steroid exposed male rats elicits aggression towards females. Horm Behav 2006;50: 410–6.
Cunningham RL, McGinnis MY. Factors influencing aggression toward females by male rats exposed to anabolic androgenic steroids during puberty. Horm Behav 2007;51: 135–41.
Cunningham RL, McGinnis MY. Prepubertal social subjugation and anabolic androgenic steroid-induced aggression in male rats. J Neuroendocrinol 2008;20:997-1005.
Delville Y, Melloni RH, Ferris CF. Behavioral and neurobiological consequences of social subjugation during puberty in golden hamsters. J Neurosci 1998;18:2667–72.
Farrell SF, McGinnis MY. Effects of pubertal anabolic-androgenic steroid (AAS) administration on reproductive and aggressive behaviors in male rats. Behav Neurosci 2003;117:904–11.
Farrell SF, McGinnis MY. Long-term effects of pubertal anabolic-androgenic steroid exposure on reproductive and aggressive behaviors in male rats. Horm Behav 2004;46:193–203.
Feinberg MJ, Lumia AR, McGinnis MY. The effect of anabolic-androgenic steroids on sexual behavior and reproductive tissues in male rats. Physiol Behav 1997;62: 23–30.
Ferris CF, Messenger T, Sullivan R. Behavioral and neuroendocrine consequences of social subjugation across adolescence and adulthood. Front Zool 2005;2:7.
Galligani N, Renck A, Hansen S. Personality profile of men using anabolic androgenic steroids. Horm Behav 1996;30:170–5.
Gentry RT, Wade GN. Androgenic control of food intake and body weight in male rats. J Comp Physiol Psychol 1976;90:18–25.
Grimes JM, Melloni RH. Prolonged alterations in the serotonin neural system following the cessation of adolescent anabolic-androgenic steroid exposure in hamsters (*Mesocricetus auratus*). Behav Neurosci 2006;120:1242–51.
Harrell WA. The effects of an aggressive model on the magnitude of extinction-induced aggression. J Soc Psychol 1973;90:311–5.
Harrison RJ, Connor DF, Nowak C, Nash K, Melloni RH. Chronic anabolic-androgenic steroid treatment during adolescence increases anterior hypothalamic vasopressin and aggression in intact hamsters. Psychoneuroendocrinology 2000;25:317–38.
Keleta YB, Lumia AR, Anderson GM, McGinnis MY. Behavioral effects of pubertal anabolic androgenic steroid exposure in male rats with low serotonin. Brain Res 2007;1132:129–38.
Kindlundh AM, Bergström M, Monazzam A, Hallberg M, Blomqvist G, Långström B, et al. Dopaminergic effects after chronic treatment with nandrolone visualized in rat brain by positron emission tomography. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:1303–8.
Kindlundh AM, Rahman S, Lindblom J, Nyberg F. Increased dopamine transporter density in the male rat brain following chronic nandrolone decanoate administration. Neurosci Lett 2004;356:131–4.
Korenbrot CC, Huhtaniemi IT, Weiner RI. Preputial separation as an external sign of pubertal development in the male rat. Biol Reprod 1977;17:298–303.
Kubala KH, McGinnis MY, Anderson GM, Lumia AR. The effects of an anabolic androgenic steroid and low serotonin on social and non-social behaviors in male rats. Brain Res 2008;1232:21–9.
Kurling S, Kankaanää A, Ellermaa S, Karlia T, Seppälä T. The effect of sub-chronic nandrolone decanoate treatment on dopaminergic and serotonergic neuronal systems in the brains of rats. Brain Res 2005;1044:67–75.
Lansford JE, Dodge KA, Pettit GS, Crozier J, Kaplow J. A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. Arch Pediat Adolesc Med 2002;156: 824–30.
Lumia AR, Thorner KM, McGinnis MY. Effects of chronically high doses of the anabolic androgenic steroid, testosterone, on intermale aggression and sexual behavior in male rats. Physiol Behav 1994;55:331–5.
Martinez-Sanchis S, Salvador A, Moya-Albiol L, Gonzalez-Bono E, Simon VM. Effects of chronic treatment with testosterone propionate on aggression and hormonal levels in intact male mice. Psychoneuroendocrinology 1998;23:275–93.
Matzel LD. Aggression in rats as a function of target location in a double alley. Am J Psychol 1984;97:519–26.
McGinnis MY, Lumia AR, Breuer ME, Possidente B. Physical provocation potentiates aggression in male rats receiving anabolic androgenic steroids. Horm Behav 2002;41:101–10.
Melloni RH, Connor DF, Hang PT, Harrison RJ, Ferris CF. Anabolic-androgenic steroid exposure during adolescence and aggressive behavior in golden hamsters. Physiol Behav 1997;61:359–64.
Nomura M, Durbak L, Chan J, Smithies O, Gustafsson JA, Korach KS, et al. Genotype/age interactions on aggressive behavior in gonadally intact estrogen receptor beta knockout (betaERKO) male mice. Horm Behav 2002;41:288–96.
Perry PJ, Kutscher EC, Lund BC, Yates WR, Holman TL, Demers L. Measures of aggression and mood changes in male weightlifters with and without androgenic anabolic steroid use. J Forensic Sci 2003;48:646–51.
Pope HG, Kouri EM, Hudson JI. Effects of supraphysiologic doses of testosterone on mood and aggression in normal men: a randomized controlled trial. Arch Gen Psychiatry 2000;57:133–40.
Primus RJ, Kellogg CK. Gonadal hormones during puberty organize environment-related social interaction in the male rat. Horm Behav 1990;24:311–23.
Ricci LA, Schwartzer JJ, Melloni RH. Alterations in the anterior hypothalamic dopamine system in aggressive adolescent AAS-treated hamsters. Horm Behav 2009;55: 348–55.
Romeo RD. Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. J Neuroendocrinol 2003;15:1185–92.
Sisk CL, Foster DL. The neural basis of puberty and adolescence. Nat Neurosci 2004;7: 1040–7.
Sisk CL, Schulz KM, Zehr JL. Puberty: a finishing school for male social behavior. Ann NY Acad Sci 2003;1007:189–98.
Skarberg K, Engstrom I. Troubled social background of male anabolic-androgenic steroid abusers in treatment. Subst Abuse Treat Prev Policy 2007;2:20.
Smith WJ, Stewart J, Pfaus JG. Tail pinch induces fos immunoreactivity within several regions of the male rat brain: effects of age. Physiol Behav 1997;61:717–23.
Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.
Thiblin I, Finn A, Ross SB, Stenfors C. Increased dopaminergic and 5-hydroxytryptaminergic activities in male rat brain following long-term treatment with anabolic androgenic steroids. Br J Pharmacol 1999;126:1301–6.
Wekerle C, Wolfe DA, Hawkins DL, Pittman AL, Glickman A, Lovald BE. Childhood maltreatment, posttraumatic stress symptomatology, and adolescent dating violence: considering the value of adolescent perceptions of abuse and a trauma mediational model. Dev Psychopathol 2001;13:847–71.
Wekerle C, Leung E, Wall AM, MacMillan H, Boyle M, Trocme N, et al. The contribution of childhood emotional abuse to teen dating violence among child protective services-involved youth. Child Abuse Negl 2009;33:45–58.
Wesson DW, McGinnis MY. Stacking anabolic androgenic steroids (AAS) during puberty in rats: a neuroendocrine and behavioral assessment. Pharmacol Biochem Behav 2006;83:410–9.
Wolfe DA, Scott K, Wekerle C, Pittman AL. Child maltreatment: risk of adjustment problems and dating violence in adolescence. J Am Acad Child Adolesc Psychiatry 2001;40:282–9.
Wommack JC, Delville Y. Repeated social stress and the development of agonistic behavior: individual differences in coping responses in male golden hamsters. Physiol Behav 2003;80:303–8.
Wommack JC, Salinas A, Melloni RH, Delville Y. Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. J Neuroendocrinol 2004;16:767–75.