



## Adolescent anabolic/androgenic steroids: Aggression and anxiety during exposure predict behavioral responding during withdrawal in Syrian hamsters (*Mesocricetus auratus*)



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### ABSTRACT

In the U.S. and worldwide anabolic/androgenic steroid use remains high in the adolescent population. This is concerning given that anabolic/androgenic steroid use is associated with a higher incidence of aggressive behavior during exposure and anxiety during withdrawal. This study uses pubertal Syrian hamsters (*Mesocricetus auratus*) to investigate the hypothesis that an inverse behavioral relationship exists between anabolic/androgenic steroid-induced aggression and anxiety across adolescent exposure and withdrawal. In the first experiment, we examined aggression and anxiety during adolescent anabolic/androgenic steroid exposure and withdrawal. Adolescent anabolic/androgenic steroid administration produced significant increases in aggression and decreases in anxiety during the exposure period followed by significant decreases in aggression and increases in anxiety during anabolic/androgenic steroid withdrawal. In a second experiment, anabolic/androgenic steroid exposed animals were separated into groups based on their aggressive response during the exposure period and then tested for anxiety during exposure and then for both aggression and anxiety during withdrawal. Data were analyzed using a within-subjects repeated measures predictive analysis. Linear regression analysis revealed that the difference in aggressive responding between the anabolic/androgenic steroid exposure and withdrawal periods was a significant predictor of differences in anxiety for both days of testing. Moreover, the combined data suggest that the decrease in aggressive behavior from exposure to withdrawal predicts an increase in anxiety-like responding within these same animals during this time span. Together these findings indicate that early anabolic/androgenic steroid exposure has potent aggression- and anxiety-eliciting effects and that these behavioral changes occur alongside a predictive relationship that exists between these two behaviors over time.

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### Introduction

The recreational use of anabolic/androgenic steroids (AAS) by adolescent teens has remained a concern for decades yet its use has risen in recent years worldwide (Harmer, 2010; NIDACapsules, 2007) despite strong evidence for negative acute and long-term physical, psychological and behavioral consequences. While the most common negative behavioral effect of AAS use is increased aggression in adult (Isacson and Bergman, 1993; Kouri et al., 1995; Kreuz and Rose, 1972; Pope, 1988; Pope and Katz, 1994; Pope et al., 2000; Strauss, 1983, 1987; Su et al., 1993) and youth populations alike (Archer, 1991; Beaver et al., 2008; Dabbs et al., 1987, 1991; Johnson, 1990; Johnson et al., 1989; Mattsson et al., 1980; Olweus, 1987; Olweus et al., 1980; Scerbo and Kolko, 1994; Schaal et al., 1996; Schalling, 1987), an increased incidence

of anxiety-related disorders is being diagnosed in AAS users (Bahrke et al., 1990; Daly et al., 2003; Johnson, 1990; Pagonis et al., 2006a, 2006b; Pope, 1988; Pope and Katz, 1994), particularly during withdrawal from AAS use (Bahrke et al., 1990; Brower, 1992, 2002; Corrigan, 1996; Kashkin and Kleber, 1989; Lindqvist et al., 2007; Malone and Dimeff, 1992; Malone et al., 1995; Perry and Hughes, 1992; Perry et al., 1990; Pope et al., 1996; Su et al., 1993). Interestingly, AAS users in many clinical studies also present with marked increases in both aggression and anxiety (Hall et al., 2005; Pagonis et al., 2006a; Pope et al., 2000; Su et al., 1993), suggesting that AAS exposure may promote the development of both negative behavioral phenotypes simultaneously. Yet, while considerable preclinical study has investigated the link between AAS use and aggression (Lumia et al., 1994; McGinnis, 2004; McGinnis et al., 2002a, 2002b; Melloni and Ricci, 2010) or anxiety (Agis-Balboa et al., 2009; Aikey et al., 2002; Ambar and Chiavegatto, 2009; Barreto-Estrada et al., 2004; Bing et al., 1998; Bitran et al., 1993; Costine et al., 2010; Fernandez-Guasti and Martinez-Mota, 2005; Koukoulas et al., 1999; Minkin et al., 1993; Ovsukova et al., 2003; Parrilla-Carrero et al., 2009; Ricci et al., 2012; Rocha et al., 2007; Rojas-Ortiz et al., 2006), no preclinical studies have

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investigated the effects of AAS administration on the temporal relationship between the expression of the aggressive- and anxiety-related behavioral phenotypes.

Here we present the first set of preclinical studies that investigate the consequence of adolescent AAS exposure on the relationship between the expression of aggression and anxiety as they present during AAS exposure and withdrawal. We hypothesized that adolescent AAS exposure would produce behavioral alterations in aggression and anxiety during both the exposure and withdrawal time periods, and that the expression of one behavior would predict the expression of the other over time. More specifically, we hypothesized that adolescent AAS-treated animals would present with high levels of aggression and low levels of anxiety during AAS exposure that would predict low levels of aggression and high levels of anxiety in these same animals during AAS withdrawal. To address these hypotheses we first investigated whether adolescent AAS exposure altered anxiety-like responding immediately (i.e., during AAS exposure) or only during withdrawal from AAS as we previously observed (Ricci et al., 2012). Next, adolescent AAS-treated animals were tested for aggression and anxiety during AAS exposure, separated into quantitatively unique groups based on their aggression level during the exposure period, and then tested for both aggression and anxiety during AAS withdrawal. Data from these animals were analyzed using between- and within-subjects statistical procedures, along with simple linear regression analyses to evaluate the predictive relationship between aggression and anxiety during adolescent AAS exposure on aggression and anxiety during withdrawal from adolescent AAS exposure.

## Methods

### Animals

Intact pubertal male Syrian hamsters (*Mesocricetus auratus*) postnatal day 21 (P21) were obtained from Charles River Laboratories (Wilmington, MA), individually housed in polycarbonate cages, and maintained at ambient room temperature (22–24 °C with 55% relative humidity) on a reverse light/dark cycle (12L:12D; lights off at 7:00). Food and water were provided ad libitum. For aggression testing, stimulus (intruder) males of equal size and weight to the experimental animals were obtained from Charles River one week prior to the behavioral test, group housed at 5 animals/cage in large polycarbonate cages, and maintained as above to acclimate to the animal facility. All intruders were evaluated and prescreened for low aggression (i.e., Disengage and Evade) and submission (i.e., Tail-up Freeze, Flee, and Fly-away) one day prior to the aggression test to control for behavioral differences between stimulus animals, as previously described in a number of our previous studies (Ferris et al., 1997; Melloni et al., 1997; Ricci et al., 2004; Ricci, 2005). Intruders displaying significantly low aggression and/or submissive postures were excluded from use in the behavioral assay. All experimental treatments and behavioral tests described below were administered during the first four hours of the dark cycle under dim-red illumination to control for circadian influences. All studies using live animals were approved by the Northeastern University Institutional Animal Care and Use Committee (NU-IACUC), and all methods used were consistent with guidelines provided by the National Institute of Health for the scientific treatment of animals.

### Experimental treatment

#### AAS treatment

Postnatal day 27 (P27) male Syrian hamsters were weighted and received daily subcutaneous (SC) injections (0.1 ml–0.2 ml) for 30 consecutive days (P27–P56) of a mixture (or “stack”) of 3 commonly used AAS (Hall et al., 2005) in doses consistent with repeated, moderate use in humans as described (see Melloni and Ricci, 2010 for a review). The AAS cocktail was composed of 2 mg/kg testosterone cypionate,

2 mg/kg nortestosterone and 1 mg/kg dihydrotestosterone undecylate (Steraloids, Newport, RI) dissolved in sesame oil (SO). As control, a separate set of P27 male hamsters received daily subcutaneous (SC) injections (0.1 ml–0.2 ml) for 30 consecutive days (P27–P56) of SO (vehicle control).

### Experimental design

On the day following the last AAS injection (P57), AAS-treated animals ( $n = 45$ ) and SO-treated controls ( $n = 22$ ) were randomly assigned to one of two counterbalanced groups and tested for anxiety-like responding using the Elevated Plus Maze (EPM) test and aggressive behavior using the Resident/Intruder (RI) test. At the completion of behavioral testing on P57, the animals were placed back into their home cage and withdrawn from AAS (or SO) for 21 days (i.e., until P77) and then again tested for anxiety and aggression.

Following behavioral tests for aggression on P57, AAS-treated animals were re-coded according to their aggressive response as compared to SO-treated control animals and then separated into No/Low (NL), Species Normative (SN) or Excessive (E) aggressive responders using inclusion criteria described in the Statistical analysis section. Animals meeting the criteria for each group of aggressive responders were analyzed using within-subjects and linear regression analyses for offensive aggression and anxiety.

In a separate set of AAS-treated animals ( $n = 30$ ) aggression and anxiety tests were performed on P57 using the EPM/RI sequence as there were no notable effects of testing sequence in Experiment 1. At the completion of behavioral testing on P57, the animals were withdrawn from AAS for 21 days (i.e., until P77) and then tested again for aggression and anxiety using the same sequence strategy. In this set of animals an array of ancillary behaviors, including social, comfort, and motor behaviors, were measured both during AAS exposure (i.e., on P57) and withdrawal (i.e., on P77) to control for nonspecific behavioral effects of adolescent AAS on behavioral responding at these two time points.

### Behavior testing

#### Aggression

Hamsters were tested for offensive aggression using the resident-intruder (RI) paradigm, a well-characterized and ethologically valid model of offensive aggression in Syrian hamsters (Floody and Pfaff, 1977; Lerwill and Makings, 1971). For this measure, a novel intruder of similar size and weight was introduced into the home cage of the experimental animal (resident) and the resident was scored for specific and targeted aggressive responses observed as lateral, flank-directed attacks as previously described (Grimes et al., 2003; Ricci et al., 2006). An attack was scored each time the resident animal would pursue and then either [1] lunge towards and/or [2] confine the intruder by upright and sideways threat; each generally followed by a direct attempt to bite the intruder's dorsal rump and/or flank target area(s). The latency to attack was defined as the period of time between the beginning of the behavioral test and the first attack the residents made towards an intruder. In the case of no attacks, latencies to attack were assigned the maximum latency (i.e., 600 s). Each aggression test lasted for 10 min and was videotaped and scored manually by two observers unaware of the hamsters' experimental treatment. Inter-rater reliability was set at 95%. No intruder was used for more than one behavioral test, and all subjects were tested during the first 4 h of the dark cycle under dim red illumination to control for circadian influences on behavioral responding.

#### Anxiety

Hamsters were tested for anxiety-related behavior using the elevated plus maze (EPM) test as in our previous study (Ricci et al., 2012). The EPM has been used extensively in rodents as a reliable test of anxiety-

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