Hormones and Behavior 56 (2009) 423–428

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Gonadal hormones modulate the display of conditioned defeat in male Syrian hamsters

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ARTICLE INFO

Article history:
Received 7 April 2009
Revised 26 June 2009
Accepted 23 July 2009
Available online 3 August 2009

Keywords:
Anxiety
Testosterone
Social defeat
Aggression
Subordination
Gonadectomy
Estradiol
Depression

ABSTRACT

It has been widely reported that gonadal hormones influence the display of aggression in Syrian hamsters; conversely, much less is known about whether gonadal hormones modulate submissive/defensive behaviors in these animals. Following social defeat, male hamsters no longer display normal territorial aggression but instead display submissive/defensive behavior in the presence of a smaller opponent, a phenomenon we have termed conditioned defeat (CD). The purpose of the present study was to examine the effect of gonadal hormones on the display of CD in male hamsters. In Experiment 1, males were castrated or sham-operated. The castrated males were significantly more submissive following social defeat relative to their intact counterparts. The increased submissive behavior in the castrated males during CD testing was particularly surprising, given the fact that they were attacked significantly less during CD training. In Experiment 2a, males were castrated and given hormone replacement. Castrated males treated with testosterone or dihydrotestosterone displayed significantly less submissive behavior following social defeat than did those treated with cholesterol or estradiol. Finally, in Experiment 2b, there was no effect of hormone replacement on aggressive behavior in non-defeated hamsters suggesting that the decrease in submissive behavior in males treated with dihydrotestosterone or testosterone is specific to being previously defeated. Taken together the data indicate that the presence of androgens reduces the display of submission in defeated male hamsters. More importantly, these findings suggest that androgens may have a protective effect against the development of depression-like or anxiety-like behaviors following exposure to an ethologically relevant stressor.

Introduction

Agonistic behavior includes all behaviors that are exhibited during social conflict, including aggressive, submissive/defensive and communicative (e.g., scent marking) behaviors. In most rodent species, agonistic behavior has been studied almost exclusively in males, as females display little aggression or fighting except in defense of their offspring; (see Lonstein and Gammie, 2002). Conversely, agonistic behavior has been widely studied in both male and female Syrian hamsters, as both display high levels of aggressive behaviors in the laboratory (Grelk et al., 1974; Huhman et al., 2003; Meisel et al., 1988; Payne, 1973; Payne, 1974; Solomon et al., 2007; Takahashi and Lisk, 1984; Whitsett, 1975).

Several factors influence the outcome of social conflict in Syrian hamsters. For example, body weight, environment and gonadal hormones are key predictors of success during agonistic interactions. In male–male interactions, heavier males dominate smaller opponents over 60% of the time (Drickamer et al., 1973; Vandenbergh, 1971). The environment in which the agonistic encounters occur is also an important factor in predicting success. More specifically, Syrian hamsters are often more aggressive when defending their own home cage compared with a neutral area (Murphy, 1976; Murphy and Schneider, 1970), although it has been shown that Syrian hamsters are still aggressive when paired outside of their home cages (Payne and Swanson, 1970). In addition, gonadal hormones influence the likelihood of "winning." For instance, intact male hamsters are more aggressive and often defeat their castrated counterparts (Payne and Swanson, 1972; Vandenbergh, 1971).

In many species, androgens facilitate aggressive behavior in males. Castration of both rats and mice reduces aggression, while androgen replacement restores aggression in both species (Albert et al., 1986; Beeman, 1947; Bevan et al., 1958; Christie and Barfield, 1979). Likewise, in Syrian hamsters there are reports of castration decreasing aggression and androgen replacement restoring aggression (Drickamer et al., 1973; Grelk et al., 1974; Payne, 1973; Payne and Swanson, 1971). There is also evidence demonstrating that aggression in adult Syrian hamsters is independent of gonadal hormones (Garrett and Campbell, 1980; Tiefer, 1970; Whitsett, 1975).

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0018-506X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2009.07.011

While much attention has been paid to the environmental and hormonal factors that may be associated with "winning," our laboratory is interested in the behavioral and hormonal consequences of "losing." Numerous studies suggest that the losers in social conflict situations exhibit many characteristics indicative of increased depression-like and anxiety-like behaviors including but not limited to, alterations in food intake and body weight, increased social withdrawal and/or submissiveness as well as decreased locomotor activity (for review; Huhman, 2006). We study a phenomenon termed conditioned defeat (CD) in Syrian hamsters. During the CD acquisition period, the experimental animal is defeated by a larger aggressor in the aggressor's home cage. On the following day, the previously defeated animal displays increased submissive/defensive behavior and increased social withdrawal when paired with a smaller opponent. This increase in submissive behavior is particularly notable because the experimental animal is tested in its own home cage and is heavier than the intruder. As mentioned previously, both home cage testing and increased body weight are key predictors in the success of agonistic encounters. Following defeat, however, the influence of these factors is offset by the previous defeat experience.

We have previously demonstrated that gonadal hormones modulate the display of CD in female hamsters (Faruzzi et al., 2005; Solomon et al., 2007), but we have not tested the hypothesis that gonadal hormones alter CD in males. The present study was designed to assess the effects of gonadal hormones on the display of CD in male hamsters. Here, we report that the presence of androgens decreases the display of submission in defeated male hamsters.

Methods

Subjects

Subjects were adult male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories) that weighed approximately 120–130 g (10 weeks) upon arrival. Hamsters were individually housed in a temperature-controlled colony room on a 14:10 h light: dark cycle with lights off at 1100 h. Additional singly housed male hamsters weighing >180 g (>6 months old) were used as resident aggressors during defeat training. We routinely use older trained resident aggressors that are larger to ensure that the experimental animals experience defeat in a "threatening" environment. Group-housed male hamsters (five per cage) weighing 110–120 g (7 weeks old) were used as non-aggressive intruders (NAI) during conditioned defeat testing. All animals were housed in polycarbonate cages (20×40×20 cm) with wire mesh tops, corn cob bedding and cotton nesting materials. Food and water were available ad libitum. All procedures and protocols were approved by the Georgia State University Institutional Care and Use Committee and are in accordance with the Public Health Service and US Department of Agriculture guidelines.

Surgery/hormone replacement

Experiment 1

All male hamsters were anesthetized deeply with sodium pentobarbitol (90 mg/kg). In Experiment 1, hamsters were castrated or sham-operated and divided into the following groups no defeat castrated ($n=8$), no defeat sham-operated ($n=7$), defeat castrated ($n=8$) and defeat sham-operated ($n=8$). All hamsters were individually housed for 4 weeks prior to behavioral testing. All hamsters were defeated one time for 15 min by a larger resident aggressor (CD training) in the aggressor's home cage. On the following day, hamsters were paired in their own home cage for five minutes with a non-aggressive intruder (CD testing).

Experiment 2a

Male hamsters were castrated and then surgically implanted (subcutaneously) with Silastic capsules containing 17-β estradiol benzoate (E_2) ($n=9$), dihydrotestosterone (DHT) ($n=9$), testosterone (T) ($n=14$) or cholesterol (C) ($n=11$). Following surgery and capsule implantation, all animals were individually housed for four weeks. Hamsters were then defeated once for 15 min by a larger resident aggressor (CD training) in the aggressor's home cage. On the following day, these hamsters were paired in their own home cage for five minutes with a smaller non-aggressive intruder (CD testing).

Experiment 2b

A separate group of male hamsters treated with E_2 ($n=9$) DHT ($n=7$), T ($n=8$), and C ($n=6$) and singly housed for four weeks served as no defeat controls who were handled and remained in their home cage until testing with a smaller non-aggressive intruder.

Hormonal capsules

E_2 capsules were made of Silastic Brand medical-grade tubing (0.078 in. i.d.×0.125 in. o.d.), 10 mm in length, sealed with Factor II 6382 RTV Silicone and Elastomer, and filled with 5 mm of 17-β-estradiol (Sigma, cat. number E1024 St. Louis, MO). T, DHT and C capsules were made of Silastic tubing (0.078 in. i.d.×0.125 in. o.d.). T capsules were 28 mm long and filled 20 mm with testosterone propionate (Sigma, cat. number T-1500, St. Louis, MO). DHT capsules were 30 mm long and filled 20 mm with dihydrotestosterone (Sigma, cat. number A-8380, St. Louis, MO) and C capsules were 30 mm long and filled 20 mm with cholesterol (Sigma, St. Louis, MO).

Conditioned defeat training/testing

All behavioral training and testing took place during the first two hours of the daily light:dark cycle. On the day of conditioned defeat training, male hamsters were transported from the colony room to the behavioral testing room. Conditioned defeat training consisted of a single resident/intruder pairing in which experimental animals were placed into the home cage of a larger resident aggressor for 15 min . During the 15-minute defeat session, experimental animals were routinely attacked by the resident aggressors and displayed submissive and defensive behavior towards resident aggressors. On the following day, a resident/intruder pairing was used in which the experimental animals were paired in their own home cage with a smaller non-aggressive intruder. All training and testing sessions were recorded on VHS tape, transferred to CD-ROM, and scored by a trained observer blind to the experimental condition using Noldus Observer (version 5) (Noldus Information Technology, Wageningen, Netherlands). The following classes of behaviors were scored as total duration in seconds: 1) nonsocial: locomotor/exploratory, self-groom, nesting, feeding, pouching, sleeping; 2) social: attend, approach, investigate, sniff, touching nose; 3) submissive/defensive: upright and side defense, rearing, flee, full submissive posture; 4) aggressive: upright and side offense, chase, attack, bite.

Hormonal assays

Nonextracted hamster serum samples were assayed in duplicate for E_2, T, and DHT using commercial radioimmunoassay kits (Active® Estradiol RIA DSL-43100, Active® Testosterone RIA DSL-4000 and Dihydrotestosterone Non Extraction RIA DSL-96100 (Diagnostic Systems Laboratories, Inc., Webster TX), respectively. All kits were validated with hamster serum. E_2, T and DHT detection ranges were 0.52–1033.76 pg/ml, 0.01–21.69 ng/ml and 1.59–1250.70 pg/ml, respectively. The specificity for the E_2 kit for 17-β estradiol was 100%, with a negligible cross reactivity (<0.01%) for testosterone. The specificity for the T kit for T was 100%, with a 5.8% cross reactivity with DHT. This kit also had insignificant cross reactivity with 17-β estradiol (<0.01%). The specificity for the DHT kit for DHT was 100%, with a 0.6% cross reactivity for T and a negligible cross reactivity (<0.01%) for E_2. Samples from each experiment were run together in one assay,