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Inhibiting influence of testosterone on stress responsiveness during adolescence

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ABSTRACT

The maturation of the hypothalamo-pituitary-adrenal (HPA) axis is a key-component of the changes that occur during adolescence. In guinea pigs, HPA responsiveness during late adolescence depends strongly on the quantity and quality of social interactions: Males that lived in a large mixed-sex colony over the course of adolescence exhibit a lower stress response than males that were kept in pairs (one male/one female). Since colony-housed males have higher testosterone (T) levels than pair-housed males, and inhibiting effects of T on HPA function are well known, we tested the hypothesis that the decrease in stress responsiveness found in colony-housed males is due to their high T concentrations. We manipulated T levels in two experiments: 1) gonadectomy/sham-gonadectomy of colony-housed males (which usually have high T levels), 2) application of T undecanoate/vehicle to pair-housed males (which usually have low T levels). As expected, gonadectomized males showed a significantly increased stress response in comparison with sham-gonadectomized males, and T-injected males had a significantly lower stress response than vehicle-injected males. Both experiments thus confirm an inhibiting effect of T on HPA responsiveness during adolescence, which can mediate the influence of social interactions. The reduction in stress responsiveness is hypothesized to have a biologically adaptive value: A sudden increase in glucocorticoid concentrations can enhance aggressive behavior. Thus, pair-housed males might be adapted to aggressively defend their female ('resource defense strategy'), whereas colony-housed males display little aggressive behavior and are capable of integrating themselves into a colony ('queuing strategy').

Introduction

Adolescence is the gradual transition from infancy to adulthood and generally considered a very vulnerable life stage (Dahl, 2004; McCormick and Mathews, 2010): Many psychological disorders manifest for the first time during adolescence (Paus et al., 2008), and mortality is disproportionately high (Forbes and Dahl, 2010). These issues may be favored by the complex interplay of changes in the brain, in the endocrine systems, and in behavior that are typical for this phase of life (Blakemore, 2008; Forbes and Dahl, 2010; Sisk and Zehr, 2005; Yurgelun-Todd, 2007). More recently, the hypothesis emerged that said changes do not only make the adolescent more vulnerable towards adverse experiences, but also may represent an opportunity for adaptation to the current social environment (Dahl, 2004; McCormick et al., 2008; Sachser et al., 2011; Williams et al., 2001).

A key component of the age-related changes is the maturation of the hypothalamo-pituitary-adrenal (HPA) axis (Romeo, 2010b), which is one of the systems that are involved in the response towards stressors. On the one hand, it appears that adolescence is a very stressful life stage almost by definition due to the variety of changes and therefore constantly threatened homeostasis (Spear, 2000). Confirming this assumption, there is a body of literature that finds prolonged or higher stress responsiveness as well as heightened anxiety behavior in various species (reviewed by Spear, 2000, and by McCormick and Mathews, 2010).

On the other hand, there are some indicators that, during this time, stress responsiveness may actually be blunted: In periadolescent mice, the HPA response to novelty is lower than in adults (Adriani and Laviola, 2000), and periadolescent rats have a lower, although prolonged, corticosterone response compared with adult males (McCormick et al., 2008). In guinea pigs, late adolescent males display a decreased stress response towards novelty (Hennessy et al., 2006), a phenomenon that is influenced strongly by the social environment: Only males that are born and reared in large mixed-sex colonies show this reduction, whereas males that have been reared under pair-housing conditions with only one female after weaning have a higher stress response (Kaiser et al., 2007). These findings suggest that the reduction in stress responsiveness might be due to the amount of social stimulation an animal receives during adolescence. In two recent studies (Lürzel et al., 2010, 2011), we tested this hypothesis by experimentally manipulating the number of social interactions and confirmed that it is indeed the

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high amount of social stimulation a colony-housed male guinea pig receives during late adolescence that causes a lowered stress response to novelty.

The question of how this influence of the social environment is mediated is not yet answered. There are two arguments that speak for androgens as mediators: First, inhibiting effects of testosterone (T) on HPA function are widely known (El Hani et al., 1980; Gaskin and Kitay, 1971; Handa et al., 1994a; Seale et al., 2004). Second, social interactions have an enhancing effect on T secretion in a variety of species (Goymann et al., 2003; Harding, 1981; Hirschenhauser and Oliveira, 2006; van der Meij et al., 2008). In guinea pigs, there is a peak in T concentrations during late adolescence in colony-housed males (Sachser and Pröve, 1988), and in adulthood, colony-housed males have higher T levels than pair-housed males (Sachser, 1990). Thus, it only stands to reason that the high amount of social stimulation received by colony-housed males increases their T concentrations, and indeed, we could recently show that T levels increase after contact to unfamiliar conspecifics of both sexes (Lürzel et al., 2010, 2011). However, all evidence for a possible relationship between high T levels and a decreased stress response has been only correlative up to now.

The present project investigates in guinea pigs whether T indeed has an inhibitory effect on stress responsiveness during adolescence. To this purpose, two independent experiments were conducted. In Experiment 1, colony-housed males were either gonadectomized (GDX) or sham-gonadectomized (SHAM) during early adolescence (before puberty). Since there is no T in GDX over the course of adolescence, they are expected to have a higher stress response than SHAM in late adolescence. In Experiment 2, pair-housed males were injected with either a T depot (TM) or with vehicle (VM). In this case, we expected TM to have a decreased stress response in comparison with VM in late adolescence.

Methods

Animals and housing conditions

The guinea pigs (*Cavia aperea* f. *porcellus*) used were descendants of a heterogeneous shorthaired and multicolored stock of 40 animals obtained from a breeder in 1975. Experimental animals were derived from two mixed-sex colonies housed in the same room. The colonies consisted of 8–10 males and 13–15 females as well as their offspring, and showed a graduated age structure ranging from approximately 1 to 19 months. Each colony was housed in a wooden enclosure of approximately 6 m^2.

All animals could be individually identified by natural markings and were housed under controlled conditions: 12:12 LD (lights on 0700 h), temperature 22 ± 3 °C. Commercial guinea pig diet (Höveler "Spezialfutter" 1070 for guinea pigs, Höveler Spezialfutterwerke GmbH & C. KG, Langenfeld, Germany) and water were available ad libitum. This diet was supplemented with hay and straw. Vitamin C was provided in the water twice per week. The floor was covered with wood shavings, and enclosures were cleaned once per week.

All experiments were authorized by the competent local authority and were approved by the animal welfare officer of the University of Muenster (reference number: 9.93.2.10.36.07.225). Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Experiment 1—Gonadectomy and stress responsiveness of colony-housed males

At the age of 30 days, which is after weaning, but before sexual maturation, experimental animals were placed from their natal into another colony and were randomly assigned to one of two groups: They were either gonadectomized (GDX; n = 9) or sham-gonadectomized (SHAM; n = 9) on day 40 of age (Fig. 1). No more than one male from each litter was assigned to the same group. Surgery was conducted under mixed anesthesia with ketamine and medetomidine (Ketamin®, Ceva Tiergesundheit GmbH, Düsseldorf, Germany, 60 mg/kg body weight IM; Domitor®, Pfizer, Berlin, Germany, 0.25 mg/kg body weight). In addition, local anesthesia was applied using a 0.5% lidocaine solution (Xylocain® 1%, AstraZeneca, Wedel, Germany, in physiological saline, 0.05–0.1 ml SC in each side of the scrotum). Effects of medetomidine were reversed using atipamezole (Antisedan®, Pfizer, Berlin, Germany, 0.75–2 mg/kg body weight SC) after completion of surgery (at least 45 min after induction of anesthesia). Postoperative analgesia was provided by carprofen (Rimadyl®, Pfizer, Berlin, Germany, 4 mg/kg body weight SC, after induction of anesthesia). Operational procedures were based on Morgenegg (1995).

Following the time schedule of our previous studies (Kaiser et al., 2007; Lürzel et al., 2010, 2011), colony-housed males were exposed to a standardized psychological stressor to assess cortisol (C) responsiveness during both early adolescence (day 55) and late adolescence (day 120; see Assessment of steroid concentrations). Additionally, basal testosterone (T) levels were measured. Furthermore, the same procedure was also conducted before surgery (day 39) to attain a basal measure before manipulation of T levels. All procedures were conducted at specified ages of the animals with a tolerance of ± 1 d, except surgery and preceding stress response test, which were conducted at specified ages ± 2 d.

Experiment 2—Application of testosterone and stress responsiveness of pair-housed males

Experimental animals were also born and reared in the colonies up to day 30, but were then rehoused with a single female of the same approximate age in 0.5 m^2 enclosures (Fig. 1). The females used as partners were colony-born and had been housed in all-female groups since the time of weaning (day 21 of age) to prevent pregnancy. Pair-housed males were randomly assigned to one of two groups: One group (TM; n = 12) was injected SC with testosterone (T) undecanoate (Nebido®, Bayer Vital GmbH, Leverkusen, 300 mg/kg body weight), a T depot, on day 80 of age, whereas the other group (VM; n = 13) was injected with vehicle (mixture of benzylbenzoate (Caesar & Loretz GmbH, Hilden, Germany) and refined castor oil (Fagron GmbH & Co. KG, Barsbüttel, Germany)). No more than one male from each litter was assigned to the same group.

During both early adolescence (day 55) and late adolescence (day 120), pair-housed males were exposed to a standardized psychological stressor to assess C responsiveness (see Assessment of steroid

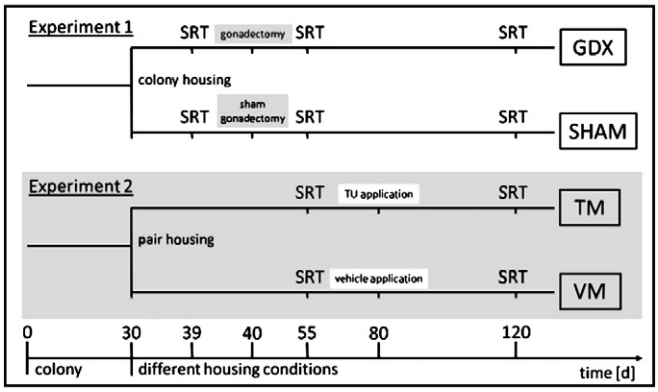

Fig. 1. Experimental design. GDX: gonadectomized males; SHAM: sham-gonadectomized males; TM: males treated with testosterone undecanoate; TU: testosterone undecanoate; VM: males treated with vehicle; SRT: stress response test. All experimental animals were born in mixed-sex colonies. On day 30, GDX and SHAM were transferred to another colony, and TM and VM were rehoused with one female only.