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# Prereproductive stress in adolescent female rats affects behavior and corticosterone levels in second-generation offspring

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**Summary** Human and animal studies indicate that vulnerability to stress may be heritable. We have previously shown that chronic, mild prereproductive stress (PRS) in adolescent female rats affects behavior and corticotropin releasing factor 1 (CRF1) expression in the brain of first-generation (F1) offspring. Here, we investigated the effects of PRS on anxiogenic behavior and CRF1 expression in male and female second-generation (F2) offspring. Furthermore, we assessed levels of the stress hormone corticosterone (CORT), a direct marker of hypothalamic—pituitary—adrenal (HPA) axis function, in PRS females and their F1 and F2 progeny. F2 offspring demonstrated decreased CRF1 mRNA expression at birth, and alterations in anxiogenic behavior in adulthood. CORT levels were elevated in PRS females and in their F1 female, but not male, offspring. In F2, CORT levels in PRS offspring also varied in a sex-dependent manner. These findings indicate that PRS in adolescent females leads to behavioral alterations that extend to second-generation offspring, and has transgenerational effects on endocrine function. Together with our previous findings, these data indicate that PRS to adolescent females affects behavior and HPA axis function across three generations, and highlight the importance of examining the transgenerational effects of stress in both male and female offspring.


## 1. Introduction

Exposure to an unpredictable, adverse environment has long-term effects on health, behavior and endocrine function, and human studies show that the effects of stress during or prior to gestation can propagate into future generations (Heijmans et al., 2008; Pembrey et al., 2014; Sigal et al., 1988; Solomon et al., 1988; Yehuda et al.,

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2008). However, human studies are not optimally suited for investigating the mechanisms underlying transgenerational transmission of stress effects.

Experiments in rodents have confirmed that the effects of trauma, particularly during dynamic periods of brain development, can be transmitted forward across several generations and impact behavior and hypothalamic—pituitary—adrenal (HPA) axis function (see Franklin and Mansuy, 2010 for Review). Several studies have shown that trauma to male rodents, which are minimally involved in pup care, is transmitted across generations and that these effects are mediated by epigenetic mechanisms (Alter et al., 2009; Dietz et al., 2011; Gapp et al., 2014a, 2014b; Morgan and Bale, 2011).

While the investigation of transgenerational transmission of trauma in male rodents has clear advantages in separating the roles played by maternal care and epigenetics, there is a dearth of studies investigating the transgenerational impact of stress in females. The effects of stress on males and females is distinct (Dalla et al., 2011; Luine, 2002), and their transgenerational effects are likely to be mediated by different mechanisms (Weinstock, 2007; Yehuda et al., 2014; Zhang et al., 2013). Furthermore, while many rodent studies focus on stress in early life, fewer studies attend to adolescence, a time period prone to significant changes in neuronal connectivity and epigenetic processes (Eiland and Romeo, 2013; Laviola et al., 2003; Niwa et al., 2013; Spear, 2000).

In a recent series of studies (Bock et al., 2014; Leshem and Schulkin, 2012; Shachar-Dadon et al., 2009; Zaidan et al., 2013), we have shown that prereproductive stress (PRS) to female rats in adolescence impacts offspring behavior, specifically in tests of anxiety and fear learning, and alters prefrontal cortex (PFC) morphology in progeny in a sex-dependent manner. We further showed that the corticotropin releasing factor receptor type 1 (CRF1), which plays an important role in the HPA axis response to stress, is altered in the PFC and oocytes of PRS female rats and in the brain of their first-generation (F1) offspring at birth and in adulthood.

While changes in the intra-uterine environment and early maternal care play a major role in the effects in F1 offspring of female rats exposed to PRS, they are less likely to explain changes observed in second-generation (F2) offspring. The influences of early life stress in male mice on the behavior of offspring has been reported for up to three generations and include an abnormal stress responses, social anxiety and deficits in social memory, with different responses in males and females (Franklin et al., 2010; Gapp et al., 2014b; Saavedra-Rodriguez and Feig, 2013; Weiss et al., 2011). The effects of adolescent female PRS on F2 offspring behavior and HPA axis function have not been studied to date.

Here, we examined the impact of chronic, mild stress in adolescent female rats, 2 weeks prior to reproduction, on behavioral measures of anxiety and aversive learning in male and female F2 offspring. Furthermore, we assessed PRS-induced changes levels of corticosterone (CORT), a direct marker of stress effects on HPA axis function (Babb et al., 2014; Oberlander et al., 2008; Rosenfeld et al., 1992), across three generations. Finally, since we previously found mRNA expression of CRF1 to be altered in the brain at birth and in adulthood (Zaidan et al., 2013), here we measured CRF1 expression in the brain of neonate and adult F2 male and female offspring.

## 2. Materials and methods

### 2.1. Animals

Female Sprague-Dawley rats were bred at the University of Haifa. They were group housed (4—5 rats per cage) in $56 \times 35 \times 19$ cm cages. Housing conditions were maintained until parturition, except during the stress procedure (see below) and included wood-flake bedding, ad lib food and water, 12 h artificial lighting during the day (07—19 h), and temperature maintained at $22 \pm 2\,^{\circ}$C. Rats were minimally handled, and soiled bedding was periodically only partially replaced, without removing the rats, so that home-cage odors, nests, etc., were minimally disrupted. The study was approved by the University of Haifa Committee on animal experimentation (197/10, 294/13).

### 2.2. Procedure

Adolescent female rats (postnatal day (PND) 42—49) were randomly divided into two groups. Females in the PRS group ($n = 20$) underwent a 7-day unpredictable stress procedure, as previously described (Leshem and Schulkin, 2012; Shachar-Dadon et al., 2009; Zaidan et al., 2013; see Fig. S1). This procedure is considered chronic and mild (Tannenbaum et al., 2002). Following the stress procedure, females in the PRS group were returned to their original cages. Control females ($n = 21$) were minimally handled except for periodic weighing and cage cleaning as described. Fourteen days following the end of the stress procedure, 7 females (3 PRS, 4 control) were sacrificed and blood samples were collected for assessment of CORT levels. Remaining animals from both groups were mated.

For mating, two females from the same group (PND63-70) were placed in each cage with an experienced adult male for 7 days. Pregnant rats were moved to same-size individual cages 3-4 days prior to parturition. Litters were raised undisturbed until PND 30. Pups were then weaned into similar size cages in groups of 4—6 same-sex rats. On PND 60 naive males and females were sacrificed, and blood samples were collected for assessment of CORT levels. Naïve daughters of PRS dams and their controls (PND60-65) were bred with non-stressed males to produce the F2 offspring of PRS and control dams (O2-PRS and O2-C, respectively). For the CORT experiment, naive male offspring of PRS and control dams were bred with non-stressed females to produce F2 offspring. On P0 (up to 24 h after birth), male and female pups from both groups were sacrificed for CRF1 mRNA expression analysis. The remaining F2 pups were raised undisturbed, except for occasional partial bedding replacement and weighing, until PND 30. Pups were then weaned into similar size cages in groups of 4—6 same-sex rats. At P60, behavioral testing was initiated, and blood samples were collected for assessment of CORT levels from behaviorally-naive rats. Brains were removed following behavioral testing, for evaluation of CRF1 mRNA gene expression in PFC and amygdala. Approximately equal numbers of males and females were tested for changes in behavior, CORT levels and CRF1 expression.