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Maternal care interacts with prenatal stress in altering sexual dimorphism in male rats

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ABSTRACT

The present study analyzes the interaction between prenatal stress and mother's behavior on brain, hormonal, and behavioral development of male offspring in rats. It extends to males our previous findings, in females, that maternal care can alter behavioral dimorphism that becomes evident in the neonates when they mature. Experiment 1 compares the maternal behavior of foster mothers toward cross-fostered pups versus mothers rearing their own litters. Experiment 2 ascertains the induced "maternal" behavior of the male pups, derived from Experiment 1 when they reached maturity. The most striking effect was that the males non-exposed to the stress as fetuses and raised by stressed foster mothers showed the highest levels of "maternal" behavior of all the groups (i.e., induction of maternal behavior and retrieving behavior), not differing from the control, unstressed, female groups. Furthermore, those males showed significantly fewer olfactory bulb mitral cells than the control males that were non-stressed as fetuses and raised by their own non-stressed mothers. They also presented the lowest levels of plasma testosterone of all the male groups.
The present findings provide evidence that prenatal environmental stress can "demasculinize" the behavior, brain anatomy and hormone secretion in the male fetuses expressed when they reach maturity. Moreover, the nature of the maternal care received by neonates can affect the behavior and physiology that they express at maturity.

Introduction

Stress caused by natural or provoked disasters (Yehuda et al., 2005) or chronic stress during gestation due to suboptimal prenatal environment (Räikkönen et al., 2011) can produce transgenerational alterations in neural development and behavior.

Many epidemiological and clinical studies in women have shown the dramatic effects of stress during pregnancy, e.g., miscarriage, low birth weight of neonates (Adams et al., 2011), high incidence of premature birth (Rondo et al., 2003), increase of neonatal pathologies and long-term diseases (Edwards et al., 1993; Gluckman et al., 2005; Jirtle and Skinner, 2007), behavioral abnormalities (Kapoor et al., 2008, 2009; O'Connor et al., 2002), fetal malformations (Blomberg, 1980), long-term effects on neural development (Talge et al., 2007) and psychiatric disorders (Bergman et al., 2007; Van Os and Selteen, 1998; Whincup et al., 2008). Because of obvious difficulties inherent in human research, the effects of prenatal stress have been examined most extensively in animal models, and especially in the rat. Although some studies have suggested that mild gestational stress does not alter maternal behavior (Pardon et al., 2000), it has been proposed that stress during pregnancy affects mother–infant relationship immediately after birth, in the preweaning period, and thus influences the developing nervous system, permanently altering behavior of the offspring. Furthermore, multiple studies have shown that gestational stress alters maternal nesting, nursing, retrieval behavior, and increases cannibalism (Del Cerro et al., 2010; Patin et al., 2002; Pérez-Laso et al., 1996, 1998; Szyf et al., 2007). We previously provided evidence that as a consequence of these alterations in the mothers' behavior toward their pups, when female rats, exposed to environmental stress, as fetuses, reached adulthood, they failed to perform appropriate maternal care with their own pups (Pérez-Laso et al., 1998). The procedure used in this study followed Ward's method by combining restraint-heat and light, also called as environmental stress applied to mothers. When referring to their offspring we use the term environmental prenatal stress (EPS).

Female offspring of stressed mothers present long-term behavioral, neuroendocrine, neural, and structural alterations in nuclei of the sexually dimorphic vomeronasal system (VNS) involved in the control of maternal behavior (MB). They present a male-like pattern in both

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induced maternal behavior and morphology of the accessory olfactory bulb (AOB). That is, those females showed a greater number of mitral cells than their gender control group, a level that did not differ significantly from the male group. They also showed increased plasma levels of ACTH and permanently elevated corticosterone (CpdB) plasma levels (Pérez-Laso et al., 2008). EPS also alters *c-fos* expression in medial preoptic area (MPOA) during induced MB (Del Cerro et al., 2010). Long-lasting deficits after prenatal stress have also been reported in sexual (Takahashi et al., 1990), social and aggressive behavior patterns of male offspring (Patin et al., 2005).

EPS produces long-lasting alterations in the hypothalamic–pituitary–gonadal axis (HPG system) and in sexually dimorphic regions of the brain (Del Cerro et al., 2010; Pérez-Laso et al., 2008; Takahashi et al., 1998). Furthermore, levels of pituitary–adrenal-hormones, released in response to stress, differ between prenatally-stressed and control animals when mature (Del Cerro et al., 2010; Pérez-Laso et al., 2008; Takahashi and Kalin, 1991). These findings suggest that exposure to environmental stress during pregnancy alters: a) development of sexual differentiation of vomeronasal structures implicated in the neural control of MB, b) hypothalamic–pituitary–gonadal and adrenal (HPG and HPA) systems and c) sexually-dimorphic behavior (Hillerer et al., 2011; Liu et al., 1997; Plyusnina et al., 2009; Sanchez, 2006; Weinstock, 2001). Recently, we reported that "appropriate" maternal care could counteract behavioral effects of prenatal stress on induced maternal behavior in female offspring when they reach maturity. However, the maternal care did not counteract neuroanatomical or hormonal alterations in those female rats (Del Cerro et al., 2010).

In the present study, we ascertained whether EPS and maternal care have a comparable effect on male offspring. In the literature there are inconsistent results ascribed to EPS-brain and behavior sexual dimorphism relationships in males. To resolve this inconsistency, we performed two experiments:

- Experiment 1: Maternal behavior of mothers that were stressed or non-stressed prenatally, toward cross-fostered or in-fostered pups (definitions: *in-fostered*: foster mothers rearing pups from donor mothers of the same treatment group, but not their own pups; *cross-fostered*: mothers rearing pups from donor mothers of a different treatment group, and thus also not their own pups).
- Experiment 2: In the six groups of male pups derived from Experiment 1, determination of the effects of the mothers' treatment (stressed vs. non-stressed) and the maternal behavior that they provided (disorganized MB vs. typical complete MB) (Numan et al., 1999) on induced MB of the male offspring when they reach maturity, as well as on their CpdB, testosterone (T), estradiol (E_2) progesterone (Prog.) plasma levels, and their AOB morphology.

Materials and methods

All experiments were carried out in accordance with the Guidelines for the use of Laboratory Animals of the European Union Research Commission and of the U.S. National Institute of Health (NIH) Laboratory Animals Guidelines.

Experimental design

Experiment 1: in-fostering and cross-fostering effects on post-partum rats' maternal behavior: stressed mothers vs. non-stressed mothers

Subjects. Fifty-six pregnant rats of the Wistar strain (Iffacredo, Barcelona — Spain), were housed in individual cages and maintained in our vivarium with food and water ad libitum, in reversed light cycle (lights off 8:00–20:00 h), temperature 20 ± 2 °C, humidity 60%.

They were divided into two groups: one group of non-stressed mothers that were left undisturbed throughout their entire pregnancy, and one group of stressed mothers that were exposed to three daily stress sessions of 45 min each, during the last week of gestation, i.e., from days 14 to 20 (at 9:00, 13:00 and 17:00 h). We used the environmental stress paradigm of Ward (1972), which involves exposure of the pregnant rat to restraint, light (2500 lx) and heat (31 ± 1 °C).

Deliveries occurred on day 21. Six hours later pups were counted, weighed and their sex recorded. Birth weights did not differ significantly between the two groups, or between female and male pups. Sex ratio was approximately 6–8 females and 4–6 males. The number of pups per litter was left intact, ranging from 9 to 12. Immediately after these observations, and within 6 h postpartum, we proceeded to cross the litters. The *in-fostering* procedure consisted of crossing the litters from one group of non-stressed mothers with a different group of non-stressed mothers; the pups of one group of stressed mothers with a different group of stressed mothers. The *cross-fostering* method was as follows: we exchanged the pups of non-stressed mothers with pups of stressed-mothers (see Fig. 1). These procedures resulted in 6 different groups of mothers: **NS**-ns (non-stressed mothers with their own pups n = 10); **NS_1**-ns_1 (*in-foster* non-stressed mothers with non-stressed pups, N = 10); **S**-s (stressed mothers with their own pups, n = 10); **S_1**-s_1 (*in-foster* stressed mothers with stressed pups, N = 12); **NS**-s (*cross-foster* non-stressed mothers with prenatally stressed pups, n = 7) and **S**-ns (*cross-foster* stressed mothers with prenatally non-stressed foster pups, n = 7).

Maternal behavior test in post-partum rats. The *in-fostering* and *cross-fostering* procedures were performed at approximately 6 h after delivery, and maternal behavior observations were initiated 42 h later. Thus, maternal behavior observations were initiated 48 h after delivery in all six groups. MB testing was carried out during 3 consecutive days. As post-partum maternal behavior follows a stereotyped sequential pattern, we set a test session length of 10 min based on previously related studies (Del Cerro et al., 2010; Fleming et al., 2002; Neumann et al., 2005; Pérez-Laso et al., 2008). We used the "MBR" software (Claro et al., 1994) to register the various maternal behavior patterns (Del Cerro et al., 1991). Observations commenced at 10:00 h under dim red light. Pups were placed by the experimenter at the cage corner opposite where the nest was located and MB pattern recording was initiated. We measured: *licking* (licking of the ano-genital area of the pups), *physical contact time* (time spent with the pups other than crouching) and *retrieval*: a) *first retrieval latency* (time elapsed before retrieving the first pup during test session) and b) *retrieval rate* (time elapsed between retrieval of the first and last pup divided by the number of pups in the litter).

Experiment 2: long-term neuroendocrine, morphological and behavioral effects of prenatal stress and maternal care on male offspring at maturity

Subjects. At 22 days of age the pups were weaned and caged into groups of same-sex individuals and experimental condition (3 or 4 per cage). In this experiment we evaluated the induced maternal behavior of two groups of female pups and six groups of male pups from the six mother groups of Experiment 1 (see Fig. 1). For this purpose we randomly selected a sample of female and male pups from each of the previous groups: **NS**-ns; **NS_1**-ns_1; **S**-s; **S_1**-s_1; **NS**-s and **S**-ns, and we tested them for MB induction when they reached maturity. The classification of our new eight groups of experiment 2 is based on the mothers' treatment followed by their own or fostering-pup condition. These groups are: non-stressed females reared by their own non-stressed mothers, NS-**nsf** (n = 15); non-stressed males reared by their own non-stressed mothers, NS-**nsm** (n = 17); non-stressed females reared by *in-foster* non-stressed mothers NS_1-**nsf_1** (n = 15); non-stressed males reared by *in-foster* non-stressed mothers NS_1-**nsm_1** (n = 17); stressed males reared by their own stressed mothers S-**sm** (n = 15); stressed males reared by *in-foster* stressed mothers S_1-**sm_1** (N = 15); stressed males reared by *cross-foster* non-stressed mothers, NS-**sm** (n = 12) and non-stressed males reared by *cross-foster* stressed mothers, S-**nsm**