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Research report

Maternal care counteracts behavioral effects of prenatal environmental stress in female rats☆

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ABSTRACT

There is extensive evidence in rats that prenatal environmental stress (PES) exposure and early postnatal altered maternal care, as a consequence of stress during gestation, can detrimentally affect the brain and behavioral development of the offspring. In order to separate the effect of PES on the fetuses from that on the behavior of the mother, in the present study, we used a cross-fostering procedure in which PES-fetuses were raised by non-stressed mothers and non PES-fetuses were raised by stressed mothers. In Experiment 1, non-stressed mothers showed significantly more maternal behavior than stressed mothers. In Experiment 2, when the female offspring from Experiment 1 reached maturity, they were tested for: (1) induced maternal behavior (MB), (2) plasma levels of corticosterone (Cpd B), progesterone (P), and estradiol (E_2), (3) number of accessory olfactory bulb (AOB) mitral cells, and (4) *c-fos* expression measured in AOB and medial preoptic area (MPOA) neurons. We replicated our previous findings that the PES group reared by their own stressed mothers, when adult, attacked the young, expressed disorganized MB and showed altered Cpd B, P and E_2 levels, plus a male-like neuro-morphological pattern in the AOB, by comparison with the non-PES group, reared by their own non-stressed mothers. By contrast, when adult, the PES group reared by non-stressed mothers showed hormonal and morphological neuronal alterations, but they displayed appropriate (full) MB. The non-PES group raised by stressed mothers also showed altered hormone levels, but showed full MB and no morphological neuronal changes. Significant differences in the AOB and MPOA *c-fos* activity, related to whether or not MB was expressed, were found in the non-PES groups, but not in the PES group reared by non-stressed mothers.

To our knowledge, this is the first study to document that adequate maternal care, early in development, can shape the subsequent expression of induced MB, overcoming neuro-morphological and hormonal alterations that are produced by prenatal environmental stress. We conclude that maternal care during early postnatal development can counteract detrimental effects of prenatal environmental stress, exerting long-lasting effects that modulate the behavioral phenotype of the offspring.

1. Introduction

Maternal behavior (MB) in rats consists of a set of behavioral patterns displayed by females that increase the likelihood of survival of the offspring [47]. There are two types of MB that we can analyze experimentally—"natural" and "induced". "Natural" MB refers to the behavior expressed by a mother toward her young immediately after parturition. "Induced" MB refers to a virgin female adopting and caring for foster pups as her own, typically several days after continuous cohabitation with them [13].

Various factors can dramatically influence MB. These include specific neurotransmitters, hormones, and prenatal environmental stress [3,64]. Previous studies have shown: (a) GABA acting as an excitatory neurotransmitter during neural development is involved in sexual differentiation of the AOB [60–62]; (b) abnormally high levels of corticosterone (Cpd B) are released during stressful events leading to abnormal estrous cycles and disrupted reproductive capacity [6,26]; (c) females that were stressed prenatally, and tested when adult for MB induction, display male-like behavior toward pups [30], which correlates with a male-typical number of mitral cells in the AOB [55].

☆ The whole experimental process and procedures of this study, have been achieved according with the European Commission rules and the American National Institutes of Health Guidelines for the Use and Care of Laboratory Animals.

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The AOB is a sexually dimorphic component of the vomeronasal pathway in the rat, in which males present a greater volume and number of neurons than females. This sexual dimorphism is established in the early postnatal period by the action of gonadal steroids [57,58,70], specifically by E_2 [53]. It is modulated by the action of neurotransmitters [62]. In addition, the AOB is involved in the tonic inhibitory control of MB in both female and male virgin rats [13]. Lesions of the vomeronasal structures, specifically the AOB [13–15] and the bed nucleus of the accessory olfactory tract (BAOT) [11,27] facilitate the expression of maternal cares to foster pups in virgin female and male rats, while lesions in the MPOA produce the opposite effect, i.e., disruption of MB [13,47].

Previously, we reported that adult females that had been exposed as fetuses to environmental stress of their mothers show a male-like pattern of both MB expression and number of mitral cells in the AOB [55]. The same females show hypothalamic–pituitary–adrenal axis (HPA) dysfunction as indicated by higher Cpd B and lower E_2 plasma levels than in control (non-PES) females. These data provide evidence of long-lasting effects of PES not only on MB, but also on morphological neuronal and physiological parameters.

The environmental stress applied to the mother is likely to affect her maternal behavior toward her pups [3,8,43]. An empirical question is whether this postpartum behavioral effect can play a significant role in disrupting the maternal behavior of her offspring when they reach adulthood. In rodents and primates, early experience acquired between birth and weaning can have profound effects on the expression of parental behavior in adulthood [2,17,18]. Furthermore, low levels of maternal care affect stress responsivity, cognition and social interactions of the offspring [29,36]. By contrast, high levels of maternal care or postnatal handling decrease the stress response of offspring and enhance learning and memory [35,41,42].

We previously proposed that long-lasting effects of PES could result from the interaction of two sets of variables, one originating in the *inner milieu*, i.e., elevated Cpd B levels in the mother exposed chronically to a stressful environment during her pregnancy, and the second from the immediate postnatal environment, i.e., disrupted maternal care resulting from stress during gestation [54,55]. These considerations led us to formulate the present study, whose main objective was to ascertain whether the long-lasting effects of PES on female rats when they were fetuses could be counteracted by their receiving adequate MB subsequently, when they were in their early postnatal period.

To address this question, Experiment 1 assessed the differences in maternal care shown by stressed vs. non-stressed mothers toward their own and cross-fostered pups. Experiment 2 ascertained, in the 4 groups of female pups generated in Experiment 1, the effects of the mothers' condition (stressed vs. non-stressed), and the maternal care that they provided (disorganized MB vs. full MB) on induced MB of the female offspring when they reached adulthood, as well as on their Cpd B, P, and E_2 plasma levels, and their AOB morphology. Since there is evidence that the expression of MB affects the activity of early genes [48], we have also quantified expression of *c-fos* in AOB and MPOA neurons after the MB acquisition test.

2. Materials and methods

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

2.1. Experimental design

Fig. 1 depicts the design of Experiments 1 and 2.

2.2. Experiment 1: effects of stress during gestation and/or cross-fostering on natural MB

2.2.1. Subjects

Thirty 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 h until 20:00 h), temperature $20 \pm 2\,^{\circ}C$, humidity 60%.

They were divided into four groups: two groups of non-stressed mothers that were left undisturbed throughout their entire pregnancy, and two groups of stressed mothers, that were exposed to three daily stress sessions of 45 min each during the last week of gestation, i.e., from day 14 to 20 (at 9:00, 13:00 and 17:00 h). We used the paradigm of Ward [71], which involves exposure of the pregnant rat to restraint, light (2500 lx), and heat ($31 \pm 1\,^{\circ}C$).

Deliveries occurred on day 21. Six hours later pups were counted, weighed and their sex was determined. Birth weights did not differ significantly among the four groups, or between female and male pups. Sex ratio was approximately 7–8 females and 5–6 males. The number of pups per litter was left intact, ranging from 10 to 16. Immediately after these observations, and within 6 h postpartum, we proceeded to cross the litters between one group of the non-stressed mothers and one group of the stressed mothers (see Fig. 1), which resulted in the 4 defined groups of mothers: **NS**-ns (non-stressed mothers with their own pups, $n = 8$); **S**-s (stressed mothers with their own pups, $n = 11$); **NS**-s (non-stressed mothers with prenatally stressed foster female pups, $n = 8$) and **S**-ns (stressed mothers with prenatally non-stressed foster female pups, $n = 8$).

2.2.2. Natural maternal behavior test

The cross-fostering procedure was performed at approximately 6 h after delivery, and MB observations were initiated at 42 h later. MB observations in all groups were thus initiated 48 h after delivery. MB testing was carried out during 3 consecutive days. Session length was 10 min, using the "MBR" software [10] to register the various maternal behavior patterns. Observations commenced at 10:00 under dim

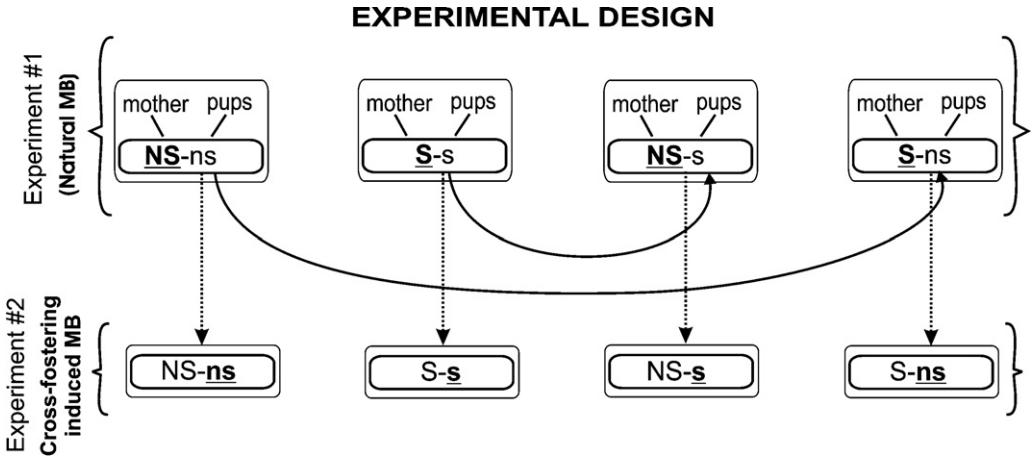

Fig. 1. Schema of methodology used in this study. Subjects in Experiment 2 are the female pups proceeding from Experiment 1, when adult.