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Combined effect of gestational stress and postpartum stress on maternal care in rats

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ABSTRACT

Variations in maternal care in the rat influence the development of individual differences in behavioral and endocrine responses to stress. This study aimed to examine the interaction between intragastric intubation during late gestation and postpartum stress, induced by pup separation, on maternal behavior and on dams' emotional state and HPA axis function. Rats received intragastric intubation of water on days 12–20 of gestation or remained untreated in their home cage (naïve dams). Pup separation was used as a model of postpartum stress. The procedure consisted of a daily separation of the dam from its litter for 3 h from PND 3 until PND 15. Pup separation was carried out in both naïve and intubated dams. The behavioral results indicate that the association of these two stressors significantly decreased arched-back nursing (ABN) and licking and grooming (LG), behaviors considered important parameters to discriminate the high quality of maternal care. Moreover, dams that received both stressors displayed less nest building and blanket nursing behaviors; no effect on the frequency of passive and total nursing was recorded. The analysis of single effects on ABN and LG, revealed that dams that underwent gestational stress induced by intragastric intubation displayed less LG, but ABN was overall unchanged. On the contrary, pup separation stress significantly increased ABN and LG upon reunion of naïve dams with their pups. Treatments per se or the association of both induced modest changes in plasma levels of allopregnanolone and corticosterone that likely did not influence maternal care. These data show that the association of a mild stress during gestation with an unfavorable experience after parturition had a significant impact on maternal care. This effect seems independent from HPA axis activation or from changes in emotional state; further studies would be necessary to ascertain the neural changes that could contribute to altered maternal behavior in stressed mothers. Moreover, these results suggest that the use of intragastric intubation during gestation would interfere with measures of drug-induced changes in maternal behavior and likely their consequences on the offspring.

1. Introduction

It is widely accepted that the maternal hypothalamic-pituitary-adrenal (HPA) axis undergoes adaptations through pregnancy essential to provide protection to negative effects of stress on pregnancy and parturition [1]. Stress hyporesponsiveness continues during lactation allowing the dam to effectively care for her developing offspring [2]. However, attenuation of HPA axis has a limited protective strength against adverse effects of stress. In fact, gestational stress has been linked to postpartum depression in humans [3,4], and to depressive-like behavior during postpartum in rats [5,6], which may be associated with a short breastfeeding duration [7] and a decrease in maternal behavior in dams [5,8]. Likewise, high levels of corticosterone (CORT) during pregnancy or the postpartum period induce depressive-like behavior and reduce maternal care in rodents [9]. These effects were observed in animal models of chronic strong stress such as 1 h restrain stress for 10 days [5] or following prolonged exposure to high levels of CORT [9]. Recently, it has been found that a mild stress during pregnancy (G17-20), due to intragastric intubation of water, may influence maternal behavior [10]. Intragastric intubation, proposed by Majchrowicz [11]

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to induce physical dependence from alcohol in adult rodents, is widely employed during the perinatal period [12], being considered an effective tool for inducing and evaluating the consequences of alcohol consumption, since the intubation procedure per se is considered devoid of long-lasting anxiety-like effects [13]. Given that the long-term outcome of an individual is greatly influenced by stress during pre- or postnatal development [14–16], it is crucial to carefully assess the effect of mild stress during pregnancy on maternal behavior. Mothers exposed to stress during gestation are in general at higher risk of encountering a more stressful environment throughout the life span, including the postpartum period [17]. An animal model used as a stressor in the early postpartum is the repeated prolonged pup separation, a protocol mainly employed to study the effects of early life stress on the offspring. In agreement with other studies [18–20], we have shown a compensatory intensification of maternal care following reunion of dams and pups separated for 3 h during the first two weeks of life [21]. The principal aim of the present study was to investigate the potential interaction between two low-level stress exposures, the first during late gestation (intragastric intubation of water on days 12–20 of gestation), and the second during the postpartum period (pup separation for 3 h on days 3–15), on maternal care by performing a wide-ranging analysis of related behaviors. Moreover, given that maternal nurturing is influenced by the emotional state of the dam, at weaning we evaluated plasma levels of allopregnanolone (AP), a positive modulator of γ-aminobutyric acid type A ($GABA_A$) receptors, and CORT, a marker of HPA axis function. We hypothesized that the pattern of secretion of these stress-sensitive neuroactive steroids during pregnancy and postpartum is altered by gestational intragastric intubation, and such alterations may influence the quality of maternal care. Indeed, in support to this hypothesis, it has been demonstrated that both AP and CORT affect maternal care [9,22].

2. Material and methods

2.1. Animals

The study was performed using males and females Sprague-Dawley rats (Charles River, Calco, Italy). All animals were housed under an artificial 12 h light (9:15 a.m.–9:15 p.m.), 12 h dark cycle (9:15 p.m.–9:15 a.m.) at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were available ad libitum. Adequate measures were taken to minimize pain or discomfort of animals, whose care and handling throughout the experimental procedures were in accordance with the European Parliament and the Council Directive of 22 September 2010 (2010/63/UE) and were approved by the Italian Ministry of Health (685/2015-PR) according to the Italian Legislative Decree no. 26 of 4 March 2014.

To obtain pregnant rats, one male 150 days old was paired with one female 120 days old. Mating was verified by the presence of the sperm cap (plug); this day was considered as gestational day 0 (GD 0). After breeding all animals were housed in group until GD 20.

2.2. Gestational stress

Twenty rats underwent gestational stress from GD 12 to GD 20 (intubated dams); once daily an intragastric cannula, connected to a 2.5 cm^3 syringe filled with water, was introduced into the stomach through the oral cavity. The control group (20 rats) was left undisturbed in their home cage (naive dams) (see Fig. 1 for a schematic of the experimental design). On GD 20 each pregnant dam was individually housed in a sawdust cage (40 cm × 60 cm × 20 cm) for parturition. All intubated females gave birth. Starting from postnatal day 2 (PND 2), all litters born within a 24 h period from mothers belonging to the same experimental group were equally distributed between males and females (5–6 for each sex). Pups were weaned at PND 21.

2.3. Postpartum stress

Pup separation was used as a model of postpartum stress. The procedure was carried out according to Plotsky and Meaney [23] and consisted of a daily separation of the dam from its litter for 3 h (from 10:30 a.m. to 1:30 p.m.) from PND 3 until PND 15. During the 3 h separation, the pups were placed in a clean cage and transferred to another room (ambient temperature at 27–30 °C) to prevent vocal communication between mother and pups. The mothers were left undisturbed in their home cage until pup reunion. The procedure was performed every day by the same experimenter. After PND 15, dams and pups were left undisturbed until weaning. Pup separation was carried out in both naïve and intubated dams (10 rats per group, respectively); two separate groups of naive (10) and intubated (10) dams, not subjected to postpartum stress, were left undisturbed with the pups and served as control (Non Pup Separated; Fig. 1).

2.4. Maternal behavior

Maternal behavior was carried out as previously described [22]. The behavior of each dam was observed for four daily 75-min observation sessions from PND 3 until PND 15. Observations occurred at a regular time each day with a session during the dark phase (8.00 a.m.) and three sessions during the light phase (9.15 a.m., 1.30 p.m. and 4.30 p.m.); thus, the first two observations took place before pup separation (which begins at 10.30 a.m.) and the last two observations were carried out after pup separation.

The distribution of the observations was based on findings that nursing in rats occurs more frequently during the light phase. For each observation session, the behavior of each mother was observed for a brief period (approximately for 5 s) 25 times, once every 3 min. Thus, every dam was observed 100 times per day (25 observations per sessions × four sessions per day = 100 observation/mother/day). Data are presented as the average frequency of each behavior across the four daily observation periods and across the 13 days of postpartum period.

The following behaviors were scored: (1) licking and grooming (LG) any pup, (2) nursing pups in passive and blanket postures (3) arched-back nursing (ABN) posture, (4) building the nest, (5) self grooming, (6) staying in the nest in contact with the pups (in nest), (7) resting with no contact with the pups (resting) and (8) eating or drinking. A detailed description of these behaviors is provided in Myers et al. (1989). Note that the behavioral categories are not mutually exclusive. For example, LG often occurred while the mother was nursing the pups. Data were analyzed as the percentage of observations in which the animal engaged in the target behavior.

2.5. Measurement of hormones levels

Within 3 h from weaning, dams were sacrificed (between 10:00 a.m. and 12:00 p.m.) by decapitation. Blood was collected from the trunk into K3-EDTA tubes, then centrifuged at 900 x g for 10 min at 4 °C; the resulting plasma was frozen at − 80 °C until use. Levels of AP and CORT were assayed in plasma from the same rats. AP was extracted from plasma as previously described [24]. The combined organic phases were dried under vacuum. The recovery (70–80%) through the extraction procedure was monitored by addition of a trace amount (6000 to 8000 cmp; 20–80 Ci/mmol) of [3H]-AP (Perkin Elmer Italia, Monza) to the plasma samples. AP levels were quantified by radioimmunoassay with a specific antibody generated in rabbit as previously described [24,25]. The limit of detection for the AP radioimmunoassay, expressed as the minimal amount of steroid distinguishable from the blank sample, was 0.01 ng. Intra- and interassay coefficients of variation ranged between 5 and 7% and between 9 and 11%, respectively.

The enzyme-linked immunosorbent assay (ELISA) was used to quantify plasma levels of CORT. ELISA was performed according to the manufacturer's instruction (CORT ELISA, IBL International, Germany)