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Effects of chronic stress during pregnancy on maternal performance in the guinea pig (*Cavia aperea* f. *porcellus*)

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ABSTRACT

Stress experienced during pregnancy can have persistent impact on the female's physiology and behaviour not only during but even beyond pregnancy. The present study aimed to evaluate such long-term effects of stress in terms of repeated strobe light exposure during early to mid gestation on behavioural aspects of mothering activities and lactational effort in lactating guinea pigs. We found that maternal behaviour was negatively affected by stress experience during pregnancy with treatment females developing a higher level of offspring-directed aggression than controls. In addition, our measure of lactational performances showed tendencies of lowered milk supply and longer pup suckling durations in stressed females. We suggest that this may represent a strategy to advance infant weaning following demanding conditions caused by chronic stress experience during pregnancy.

1. Introduction

In mammals, behavioural and energetic investment into reproduction expands after birth, with especially the time of lactation being of high costs to the female. These costs exceed energetic expenditures of gestation (Clutton-Brock et al., 1989; Loudon and Racey, 1987) and have to be balanced against the female's capacity. Even though offspring quality and subsequent reproductive success are expected to increase with maternal investment, particularly in poor environments with heightened levels of physical demand, the optimal extent of maternal effort is limited by requirements of self-maintenance (Mauck and Grubb, 1995).

Stress experienced during pregnancy stimulates changes in the female's physiology and behaviour that may persist not only during pregnancy but even beyond parturition. Stressors acting on pregnant mice, for instance, altered their emotional reactivity to stress up to one month after actual exposure (Darnaudéry et al., 2004). In the rat and mouse, short or chronic periods of stress during gestation permanently affected the pregnant and lactating female's endocrine state and body weight (Baker et al., 2008; Heiming et al., 2011). In addition, there are consistent reports of perturbed maternal care-giving activities in laboratory rodents exposed to stress during pregnancy (e.g. Champagne and Meaney, 2006; Meek et al., 2001; Patin et al., 2002; Smith et al., 2004), which in turn have been linked to heightened stress reactivity in their progeny (Baker et al., 2009; Champagne and Meaney, 2006; Smith et al., 2004).

In the present study, we aimed to assess the effects of stress exposure during pregnancy on maternal care during lactation in terms of maternal behaviour and milk yield. We used the guinea pig (*Cavia aperea* f. *porcellus*) in this study because effects of maternal subjection to social instabilities or non-social stressors have been widely explored in this species (e.g. Cadet et al., 1986; Kaiser et al., 2003a,b), though little is known about the lasting effects of stress experience during pregnancy on maternal performance during lactation.

2. Materials and methods

All husbandry and experimental procedures were approved by the institutional ethics committee and the Austrian Federal Ministry of Science and Research (GZ 68.205/0211-II/10b/2008).

2.1. Animals and housing conditions

In this experiment, short-haired, multicoloured domestic guinea pigs that could be identified by individual coat patterns were used. Fourteen female subjects aged between 3 and 6 months were randomly assigned to treatment or control group. In addition,

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7 male guinea pigs of similar age were used for breeding. Body weight was not statistically different between groups (Student's *t*-test, females: $t_{12} = 0.310$, $p = 0.762$; males: $t_5 = -0.654$, $p = 0.542$). All but three females were primiparous. Primiparous dams were present in both groups and our raw data on maternal behaviour showed an inter-individual variation in maternal care that appears to be independent of parity. All individuals were housed in the same room under standardised conditions with an average room temperature of $22.7 \pm 0.7\,^{\circ}$C, a relative humidity of 50% and a 12:12 h light–dark cycle with lights on at 7.00 h. Except for stress applications, all experiments were carried out in the maintenance room. Subjects were kept in groups of two until mating. For this purpose, a male was introduced to the females' cages (85 cm × 47 cm) and left throughout pregnancy. In the guinea pig, the stage of estrus cycle can be determined by visual inspection of the vaginal membrane, and for the purpose of stress application (see Section 2.2) cycle lengths of females were previously recorded for at least four weeks to estimate the time of conception. As however cycles of females housed together varied, up to seven days passed between introduction of the male and mating. Mating was either directly observed or a copulatory plug was present. About three days before the expected delivery, females were removed from their groups and housed individually in maternity cages (85 cm × 47 cm) to be able to deliver undisturbed. We determined the time of separation on the basis of the day of mating and the widening of the female's pelvis that can be used as indicator for upcoming birth. Pups were weaned at the age of 21 days.

Water was available ad libitum. Daily, each individual received 15 g of pelletised food for guinea pigs (Altromin 3123, Altromin GmbH, Lage, Germany), 40 g of fresh food (vegetables and fruits), and a handful of hay (~35 g). From day 45 of gestation until the end of lactation, each female was given an additional 20 g of food pellets for increased energetic requirements. Each pup received 10 g pelletised food plus 10 g fresh food.

2.2. Stress procedure

A detailed description of the stress procedure can be found in Schöpper et al. (2011). Briefly, females assigned to the treatment group ($n = 7$) were stressed using high-frequency strobe light (Mini-Flash DK 0-11, distributed by Conrad Electronic, Austria) in an unfamiliar dark room. The stressor was applied for 3 h in two blocks per day (9.00 to 11.00 h and 16.00 to 17.00 h) starting 7 days prior to mating (day −7) followed by the day of mating (day 0 of gestation) and day 7, 14, 21, 28, 35 and 42 of gestation (gestation length = approximately 68 days). Control females were left undisturbed in their home cages except for maintenance and sampling procedures (see Schöpper et al., 2011).

This stress procedure evoked a significant short-term increase in cortisol secretion but long-term down-regulation of the hypothalamic–pituitary–adrenal (HPA) axis in treatment females (Schöpper et al., 2011). In addition, reduced body weight gain was observed in stressed females during pregnancy, while reproductive output at time of birth in terms to litter size, birth weight or sex-ratio remained unaffected (Schöpper et al., 2011).

2.3. Maternal behaviour

Treatment as well as control mothers and their offspring were filmed in their maternity cages on postnatal days 1, 3, 6, 14 and 21 in two 45-min observation periods, one between 7.30 and 9.00 h, the other between 14.00 and 15.30 h. Several indicators were used to estimate birth so that continuous observations over an extensive time period were avoided and we still succeeded to directly observe all females used for measurements of maternal performance giving birth. At least four hours passed between birth of pups and first filming on day 1 postnatally. Filming usually started in the morning, except in the case of females giving birth between 3.30 and 10.00 h which were first filmed in the afternoon of the day of parturition and in the morning of the following day. Thirty minutes prior to filming, the shelter hut, which was provided in each cage, was removed and the cage was placed on the floor of the maintenance room to allow filming from above. Videos were recorded and further analysed using the software program "The Observer 2.0" (Noldus Information Technology bv, Wageningen, The Netherlands). Behaviour of the mother was measured by continuous recording (after Altmann, 1974). Duration or frequency of the following elements of maternal behaviour (following the definitions of Albers et al., 1999; Hennessy and Jenkins, 1994; Rood, 1972; Schiml and Hennessy, 1990) were recorded: (1) *grooming*: the mother exhibits nibbling or licking movements with her mouth on the face or body of an offspring (in seconds); (2) *anogenital licking*: maternal licking of the anogenital region of the young, thereby stimulating urination and defaecation (in seconds); (3) *nursing* summarised: *crouching*, the mother displays a flexed ventrum and arched back with the front legs extended to enable access to the teats (in seconds); *passive nursing*, the mother adopts passive postures (e.g. lying beside the pups) while nursing (in seconds); (4) *physical contact* between the bodies of the mother and one or more pups (in seconds); (5) *aggressive behaviour*: summation of pushing, boxing and causing flight by the mother (frequency in 90 min); (6) *leaving pup*(*s*): the mother moves away from her pup(s) so that distance becomes ≥ one body length (frequency in 90 min).

Two subjects were excluded from measures of maternal behaviour and lactation performance as one female was accidentally mated postpartum and due to missing records.

2.4. Lactational performance

Milk yield of treatment and control females was measured using a method described by Laurien-Kehnen and Trillmich (2003). On postnatal days 4, 8 and 12, the litter, its size varying between 1 and 5 pups, was separated from its mother for 1.5 h (from 11.15 to 12.45 h), during which they were kept in bedded boxes without access to water and food. Afterwards, body weight of each pup was determined on a precision scale (RS 232, Radwag GmbH, Hilden, Germany) with an accuracy of 0.01 g and pups were placed back to their mothers into the maternity cage. Also here, pups were prevented from ingesting water or food. During the following period of 30 min, the duration of suckling of individual pups was continuously registered by an experimenter present in the room. Suckling was recorded when a pup positioned its snout at its mother's ventrum for longer than 30 s so that milk could be drunk (after Schiml and Hennessy, 1990). At the end of the observation period, pups were removed and their body weights measured again. The difference in the individual's body weight was considered the amount of milk yield per pup. It has to be stated that except for lactation day 4, due to faeces and urine disposal by pups during the course of observation, most measures on milk yield revealed higher body weight loss than weight gain by milk intake.

2.5. Statistics

Between-group comparisons of maternal behaviour were performed by applying a linear mixed effects (LME) model to adjust for repeated measurements, with group (treatment versus control), time, and litter size as fixed factors, and female subjects as random factor. Non-significant interactions were stepwise excluded from the model; *F*-values with degrees of freedom and corresponding *p*-value of the resulting models are presented. Variables were