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Research report

Maternal separation and maternal care act independently on the development of HPA responses in male rats

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ABSTRACT

Postnatal manipulations such as brief (early handling, EH) and long, daily mother–offspring separations (maternal separation, MS) in rats are used to study the mechanisms underlying developmental plasticity of stress and fear responses, and to model stress-related disorders in humans and in non-human animals. Current evidence suggests that, compared to non-handled rats, EH reduces hypothalamic-pituitary-adrenal (HPA) reactivity in the adult offspring through stimulating increased levels of active maternal care. In contrast, despite a similar increase in active maternal care, MS does not reduce HPA reactivity, thus suggesting that long mother–offspring separations may counteract the effects of increased active maternal care. We therefore attempted to selectively manipulate levels of active maternal care and durations of mother–offspring separations in neonate rats. Rat pups were exposed to different combinations of EH and MS from postnatal day (PND) 2 to 10 using a split-litter design. Maternal behaviour was recorded from PND 2 to 8 and behavioural and endocrine responses to stress were studied in adult male offspring. Low levels of maternal care combined with long mother–offspring separations increased HPA-reactivity compared to both high maternal care combined with long mother–offspring separations and low maternal care combined with brief separations. These findings further support the hypothesis that active maternal care and long mother–offspring separation act independently, and exert opposing effects, on adult offspring's HPA responses, but that increased maternal care may buffer the adverse consequences of long separations.

1. Introduction

Early life experiences have long been known to exert strong influences on behavioural and brain development in animals and humans (e.g. [17,19–21]). In humans adverse early life events such as physical or emotional neglect or violence directly relate to the emergence of psychiatric disorders later in adulthood [13,14]. Such pathological outcomes often depend on a dysregulation of the hypothalamic-pituitary-adrenocortical (HPA) system and on the subsequent abnormal regulation of circulating levels of stress hormones [4]. Several animal models used to study the mechanisms underlying neonatal plasticity of the HPA-system and to model stress-related human disorders, are based on rats [1,3,6,7,12,19–21,29,32,36,39], yet the factors mediating HPA-axis development in rats have remained elusive [23,25]. A better understanding of environment-dependent plasticity of the HPA-system in rats could advance our knowledge of experience-dependent plasticity of brain and behaviour and help improve the validity of rat models of stress-related disorders.

Almost 50 years ago, Levine [18,19] provided the first experimental evidence that the capacity of rats to cope with stress is modulated by early experience. Thus, adult rats separated daily from their mothers for brief periods (3 min; early handling, EH) during the first 3 weeks of life showed reduced adrenal gland weights 24 h after a single glucose injection [20]. A few years later, Denenberg et al. [9] proposed that these effects might be mediated by changes in maternal behaviour induced by EH (see also [41]). However, only quite recently has this hypothesis been confirmed experimentally (reviewed by [29]; see also [38]). Thus, EH was found to stimulate active maternal care, and similar to EH, spontaneously high levels of active maternal care were associated with reduced behavioural and endocrine stress responses in the adult offspring [10,22]. These changes in HPA-reactivity seem to depend on alterations in DNA methylation and chromatin structure induced by variations in active maternal care [49].

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In contrast, longer periods of daily maternal separations (MSs) (3–6 h) produced far less consistent results. Compared to undisturbed controls (non-handling), MS was sometimes found to increase behavioural and endocrine stress reactivity (e.g. [2,27,35]), while in other cases no significant effects (e.g. [6,15,21,23]) or even opposite effects were detected (see [28] for reduced fearfulness and [31] for reduced HPA responsiveness). Despite these inconsistencies, it has been proposed that MS deprives pups of active maternal care, which, in turn, induces increased stress and fear responses in the adult offspring [29]. However, we observed that MS induced elevated levels of active maternal care similar to EH, despite the daily 4 h mother–offspring separation [23]. This was due to a general up-regulation of active maternal care throughout the day similar to EH, and massively elevated levels of maternal care in the hours following reunion of dam and pups, which fully compensated for the lack of maternal care during the 4 h separation. However, unlike EH offspring, MS offspring showed elevated stress and fear responses similar to undisturbed controls [23]. This dissociation in the effects of EH and MS on maternal care and the adult offspring's stress and fear responses indicates that the total amount of active maternal care cannot fully explain the effects of these neonatal manipulations on offspring phenotype. Thus, besides levels of active maternal care, other factors associated with long mother–offspring separations involved in MS must contribute to the effects of MS on later stress and fear responses in the offspring. In line with Pryce and colleagues [38,39], we proposed that MS may affect pup homeostasis by e.g. reduced food intake, altered diurnal distribution of nursing bouts, or a transient decline of body temperature [23]. We recently provided additional evidence that maternal care is not the unique mediator of environment-dependent plasticity of HPA responses in the adult offspring [23,52]. In order to modulate nest attendance through environmental modification, we provided rat mothers with low, moderate and high foraging demands and studied the effects of this manipulation on maternal behaviour and on adult offspring stress and fear responses [24]. Compared to both low and high environmental challenges, moderate foraging demands reduced neuroendocrine and fear-related behaviour in the adult offspring and increased the frequency and duration of nest excursion. High environmental challenges had similar effects on nest excursion but no effect on adult HPA responses. Thus, whereas nest attendance explained the different adult stress and fear responses between rats reared under low and moderate challenges, the same factor failed to explain the observed difference between moderate and high challenges.

The aim of the present study was to further examine the relative contributions of active maternal care and duration of mother–offspring separations to the development of stress and fear responses in rats. To this end we attempted to selectively manipulate (i) the duration of mother–offspring separations; (ii) the level of active maternal care, and (iii) to create rearing conditions that differed along these two factors. We thus adopted a split-litter design [44–48] in which animal facility rearing (AFR) conditions, EH and MS were combined in specific ways to achieve the experimental dissociation of the duration of mother–offspring separation and levels of active maternal care. The ultimate goal was to study the relative contribution of each of these two factors on the development of HPA responses.

2. Materials and methods

2.1. Animals/housing conditions

Pregnant Lister Hooded females (purchased from Harlan, NL-5960, Horst, The Netherlands) were housed in standard polycarbonate cages (59.0 cm × 38.5 cm × 20.0 cm) with sawdust bedding and *ad libitum* water and rodent pellets (Universal feed 3430, Moulin Kilba SA, Kaiseraugst, CH). They were maintained on a reversed 12:12 h light:dark cycle (lights on at 19:00 h) with temperature at 21 ± 1 °C and relative humidity of 55 ± 5%. They were then inspected daily at 09:30 h for delivery and day of birth was designated as postnatal day 1 (PND 1). On PND1 pups were marked by toe clipping and mothers and pups were randomly allocated to treatment groups as described below (N = 8 per group). At PND 21, offspring were weaned into same-sex groups of four littermates. One week prior to testing, all offspring were re-housed in pairs. Adult rats were used only once. To avoid litter effects, only one male subject per treatment per test was used in adulthood. The experiment was run in two replicates (N = 4 per group per replicate). All experimental manipulations were conducted under experimental permit in accordance with the Swiss Animal Protection Act (1978).

2.2. Experimental treatments

To induce dissociation in the level of maternal care and the duration of mother–offspring separations, the following treatments were applied from PND 2 to 10 (see Fig. 1 for a graphical representation):

Animal facility rearing (AFR): dam and pups were subjected to standard housing conditions, involving the provision of food and water and cage cleaning once a week. Due to reduced litter size one dam was removed from the study (N = 7).

Maternal separation/maternal separation (MSMS): at 7:30 the dam was briefly removed from the home cage and at least four pups (two males and two females) were placed in an adjacent cage for 4 h (MSms offspring); at 9:30, the dam was again briefly removed from the home cage and the remaining part of the litter (two males and two females) was placed in an adjacent cage for 4 h (msMS offspring); at 11:30 MSms pups were returned to the home cage and at 13:30 msMS pups were returned to the home cage (N = 8).

Maternal separation/early handling (MSEH): at 7:30 the dam was briefly removed from the home cage and all pups were placed in an adjacent cage; 15 min later, part of the litter (two males and two females) was returned to the home cage (msEH offspring), while the remaining part of the litter (at least two males and two females; MSeh offspring) remained in the separation cage; at 11:15 the dam was again briefly removed from the home cage and msEH pups placed again in the separation cage for another 15 min; at 11:30 both MSeh and msEH pups were returned to the home cage (N = 8).

Animal facility rearing/early handling (AFREH): at 7:30 the dam was briefly removed from the home cage and part of the litter (at least two males and two

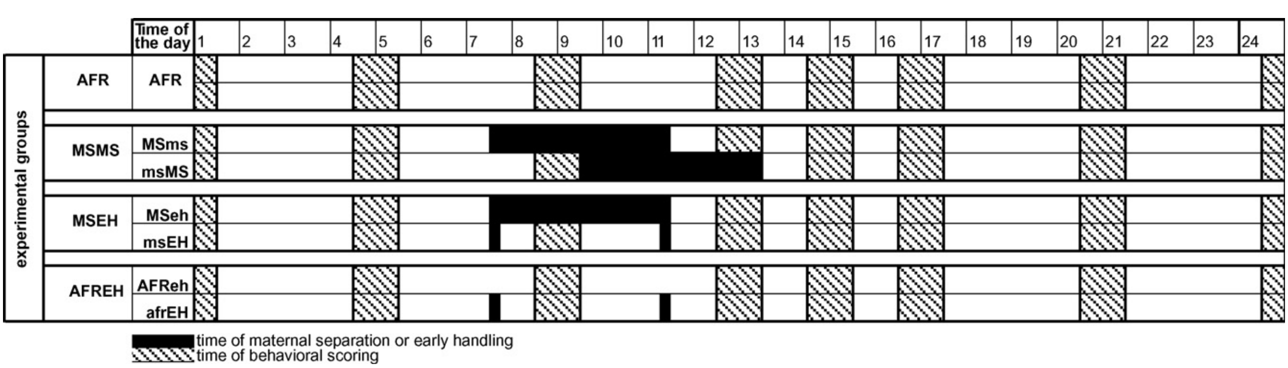

Fig. 1. Graphical representation of the neonatal manipulations and of sampling times of maternal behaviour. In the uppermost row the time of the day is reported (12 h light:dark cycle, lights on at 19:00 h). Black insets indicate the time and duration of separation for each group of littermates, while grey columns indicate the time of behavioural scoring.