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Research Report

Differential development of stress system (re)activity at weaning dependent on time of disruption of maternal care

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ABSTRACT

Maternal deprivation, a separation of mother and pups for 24 h in the first weeks of life has long-lasting consequences for the glucocorticoid stress system in rats. We examined in male CD1 mice whether the postnatal day (pnd) of deprivation determines the (re)activity of the stress system at weaning under basal and novelty stress conditions. Maternal deprivation was only effective when applied within the stress hypo-responsive period (SHRP) between pnds 1 and 12, but not on pnd 13. Maternal deprivation (i) early in the SHRP (pnd 3) resulted in lower hippocampal GR mRNA expression together with a prolonged corticosterone response to stress; while (ii) late in the SHRP (pnd 8) the amplitude of the ACTH response to stress was enhanced. (iii) Strikingly, the effects of the double deprivation (pnds 3 and 8) were not additive: sustained, stress non-responsive high plasma ACTH concentrations with corticosterone indistinguishable from control animals coincided with a lower expression of hippocampal MR and GR mRNA. These results present species-specific effects (mouse versus rat) of an adverse early life event on HPA axis regulation at weaning. A subsequent deprivation experience interferes with the effects of earlier deprivation. We conclude that the developmental stage of the organism determines the vulnerability for the detrimental effects of maternal deprivation and the organization of the stress system in adolescence.

1. Introduction

An undisturbed development of the brain, and in particular of systems involved in stress regulation and adaptation, is essential for normal functioning of an organism during adulthood. In humans traumatic early life stress, such as parental separation, childhood sexual or physical abuse, or preterm birth, has been associated with mood and anxiety disorders (Ehlert et al., 2001; Heim et al., 2000a,c), specifically with (juvenile) onset of major depressive disorder (Jaffee et al., 2002; Kendler et al., 1999). Adult patients suffering from major depressive disorder who had experienced early life stress show persistent hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and of the autonomous nervous system, as well as an increased sensitivity of these systems to stress (Checkley, 1996; Heim et al., 2001, 2000b).

In rodents, a disturbance of normal development during the so-called stress hypo-responsive period (SHRP) has been shown to alter both endocrine and behavioural functions. The SHRP from postnatal days (pnd) 1–12 in mice (Cirulli et al., 1994; D'Amato et al., 1992; Schmidt, Enthoven, van der Mark, Levine, de Kloet, and Oitzl, 2003; Schmidt et al., 2002) is characterized by low basal corticosterone concentrations and an inability of mild stressors to induce an endocrine response (postnatal day 4–14 in

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rats; Levine, 1994; Rosenfeld et al., 1992; Sapolsky and Meaney, 1986; Schapiro et al., 1962). Separating mother and pups for 24 h during the SHRP, *i.e.*, maternal deprivation, activates the HPA axis in both rats and mice (Cirulli et al., 1994; Levine, Huchton, Wiener, and Rosenfeld, 1991; Rosenfeld et al., 1991; Schmidt et al., 2002; Stanton et al., 1988; van Oers et al., 1998) and, as studied in rats, also leads to a number of long-term changes in HPA axis activity and reactivity to stress (Suchecki et al., 2000; Sutanto et al., 1996; van Oers et al., 1997; Workel et al., 2001).

Separation of mother and pups in rats in various paradigms or at different ages during the SHRP differentially affected HPA axis responsiveness (Lehmann et al., 1999, 2002; Penke et al., 2001; Pryce and Feldon, 2003; van Oers et al., 1997, 1998). van Oers et al. (1997) already called these effects "paradoxical", but they most probably depend on the developmental stage at the time of deprivation. We recently demonstrated in the mouse that the low peripheral activity at normal HPA axis functioning during the SHRP was accompanied by a high level of dynamic changes in mRNA expression profiles of several central components of the HPA axis (Schmidt et al., 2003).

Considering these dynamic changes during normal HPA axis development (Schmidt et al., 2003), we hypothesize that the age of the pups at separation from the dam will determine the long-term consequences of maternal deprivation. Therefore, we maternally deprived CD1 pups at specified days during the first two weeks of postnatal life. Mice of the control group were not disturbed, except for adding some new sawdust at approximately pnd 20. We used a single 24 h maternal deprivation early (pnd 3) and late (pnd 8) during the SHRP and at pnd 13, just outside the SHRP. To assess whether repeated maternal deprivations will result in even more severe effects we also deprived mice twice for 24 h at pnds 3 and 8. At weaning (pnd 28) we tested the basal activity and the stress-induced reactivity of the HPA axis by subjecting each mouse to a novel cage. mRNA expression of central markers of HPA activity were measured in hippocampus, the paraventricular nucleus of the hypothalamus (PVN) and amygdala. Blood plasma concentrations of corticosterone and ACTH were estimated at several time points. The presented data are in agreement with our hypothesis and underline the importance of the pup's age within the mouse SHRP when subjected to maternal deprivation.

2. Results

2.1. *Corticosterone*

Under basal conditions all groups showed similar low corticosterone concentrations. Novelty induced a stress response in all groups (main effect of time ($F(3,159)40.10$, $P<0.001$), demonstrated by elevated corticosterone levels at 10 and 30 min. After 120 min, pnd 3 deprived animals still showed significantly elevated corticosterone, whereas corticosterone of the other groups had returned towards basal levels ($P<0.05$ versus all other groups). A main treatment effect passed statistical significance ($F(4,159)2.23$, $P=0.07$). Strikingly, though there was no main interaction observed between time and treatment ($F(12,159)0.79$, $P=0.66$) the additional deprivation at pnd 8 did seem to abolish the effects induced by a maternal deprivation at pnd 3 (pnds 3&8 versus pnd 3 deprived animals) (Fig. 1).

2.2. *ACTH*

We observed a main effect of time (ANOVA: $F(3,159)21.56$, $P<0.001$) and an interaction between time and treatment ($F(12,159)1.87$, $P<0.05$) indicating the differential time course of the groups. Except for pnds 3&8 deprived animals all groups were able to elicit a stress response upon placement in the novel environment. Already under basal conditions pnds 3&8 deprived animals had significantly higher ACTH concentrations ($P<0.05$) than all other groups. Moreover, the mice sustained this high ACTH level without further increase in response to novelty. Furthermore, pnd 8 deprived animals showed the highest ACTH concentrations 30 min after novelty ($P<0.05$ versus control) (Fig. 2).

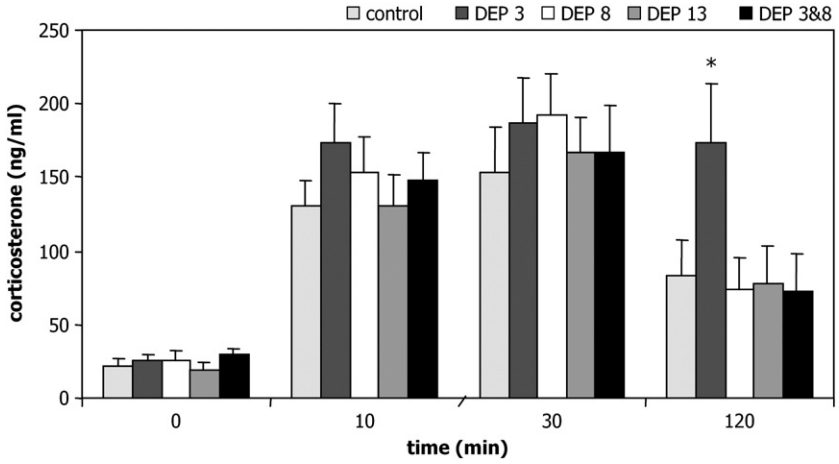

Fig. 1 – Plasma corticosterone concentrations (ng/ml) at 28 days of age: basal (t=0 min) and at 10, 30 and 120 min after exposure to a clean novel cage. Mouse pups had been deprived from their mother for 24 h once at either pnd 3 (DEP3), 8 (DEP8) or 13 (DEP13); or in a combination of pnds 3 and 8 (DEP3&8). Control mice remained undisturbed throughout their development. Data represent mean ± S.E.M., *P<0.05 versus all other groups at t=120 min.