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Maternal deprivation by early weaning increases corticosterone and decreases hippocampal BDNF and neurogenesis in mice

Takefumi Kikusui [a,b,*], Sozo Ichikawa [a], Yuji Mori [a]

[a] Veterinary Ethology, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[b] Companion Animal Research, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229-8501, Japan

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Summary We previously demonstrated that early weaning increases anxiety and neuroendocrine stress responses in rats and mice. In addition, early-weaned mice show precocious myelin formation, especially in the amygdala, suggesting that these mice are vulnerable to psychological stress. In the present experiments, we examined corticosterone response after early weaning and how early weaning affects hippocampal neurotrophic factor and neurogenesis, which have been linked to depressive behavior in human and animals models. When the mice were weaned at PD14, both male and female mice showed higher corticosterone levels up to 48 h after weaning. In contrast, after standard weaning, corticosterone levels returned to the baseline within 2 h. Early-weaned males, but not females, had less brain-derived neurotrophic factor (BDNF) protein in the hippocampus at 3 weeks of age than standard-weaned mice. Neural stem cells were labeled with bromodeoxyuridine (BrdU) injections at 2, 3, or 5 weeks of age, and assayed at 3, 5, and 8 weeks of age, respectively. Early-weaned males had fewer BrdU immunoreactive cells in the dentate gyrus at 3, 5, and 8 weeks. In early-weaned females, fewer BrdU-positive cells were observed only at 5 weeks. Double-staining with BrdU and the neuron markers NeuN and Tuj1 demonstrated that neurogenesis was lower in early-weaned mice at 5 weeks of age. These results suggest that lack of mother—infant interaction during the late lactation period leads to an increase in corticosterone synthesis for 2 days and a decrease in BDNF synthesis in males; moreover, this lack of interaction transiently inhibits hippocampal cell proliferation and survival in both males and females, although the effects were more pronounced in males.

1. Introduction

Mother—pup interactions during the early stages of life are important for neurobehavioral development (Plotsky and Meaney, 1993; Caldji et al., 2000; Liu et al., 2000). Social stress in early life, such as neglect in humans and maternal

* Corresponding author at: Companion Animal Research, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229-8501, Japan. Tel.: +81 42 769 1853; fax: +81 42 769 1853.
E-mail address: kikusui@azabu-u.ac.jp (T. Kikusui).

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deprivation in rats, is associated with changes in behavioral and physiological responses to stress in adulthood and susceptibility to psychopathology in later life.

In the normal development process of rats, pups are able to eat, maintain body temperature, and evacuate by themselves from 13 days of age (Plaut et al., 1974). From this time point and continuing through the weaning period, the neuroendocrine system is sensitive to stressors (Levine et al., 1991). After weaning, a key event marking the end of the early developmental stage, offspring must become nutritionally and behaviorally independent from their dam. Thus, it is highly likely that the weaning process influences the physiological and neurobehavioral development of rat pups. In support of this postulate, we and other researchers have identified behaviors that are influenced by early weaning (Janus, 1987; Terranova and Laviola, 2001). For example, precocious weaning of rats and mice enhances anxiety and aggression for a long period of time (Nakamura et al., 2003; Kikusui et al., 2004; Kanari et al., 2005), and results in decreased play-fighting behaviors (Shimozuru et al., 2007), suggesting that the parent–pup interaction during the later lactation period is important for behavioral development (Kikusui et al., 2005). Moreover, early-weaned male rats have higher autonomic responses to novel stressors (Ito et al., 2006) and early-weaned mice exhibit an increased stress–endocrine response to mild stress (Kikusui et al., 2006). Therefore, early weaning may alter the development of the neural circuits for the stress response.

In addition to behavioral changes, neurochemical development of the brain is also affected by maternal care. For example, animals exposed to brief periods of handling daily for the first 3 weeks of life have higher glucocorticoid receptor concentrations in both the hippocampus and the frontal cortex (Meaney et al., 1985). Furthermore, maternally deprived rats exhibit suppressed adult neurogenesis (Mirescu et al., 2004) and decreased brain-derived neurotrophic factor (BDNF) in the hippocampus (Liu et al., 2000). Increased neurogenesis in the hippocampus in response to antidepressant treatments (Malberg et al., 2000) or decreased neurogenesis due to irradiation of the hippocampus (Santarelli et al., 2003) is observed in parallel with behavioral changes, suggesting that neurogenesis in the hippocampus is related to anxiety/fear behavior. Regarding the effect of BDNF on behavior, mice genetically deficient in the BDNF-TrkB system fail to show antidepressant behavioral outcomes, suggesting that hippocampal BDNF modulates neurogenesis and behavior in the mice (Saarelainen et al., 2003). In support of this hypothesis, BDNF itself causes antidepressant-like behavioral changes (Shirayama et al., 2002). Recently, Tsankova et al. (2006) demonstrated that chronic social defeat decreases BDNF expression in the hippocampus through DNA modification, and can be recovered with antidepressant treatments (Tsankova et al., 2006).

In this study, we first investigated the influence of early weaning on HPA activity, by assessing circulating corticosterone, which would be the key factor inducing neurochemical changes in the early-weaned mice. In our previous study (Kikusui et al., 2006), we demonstrated that early weaning alters glucocorticoid receptor expression in the hippocampus, implying that early weaning results in a decrease in neurogenesis and BDNF expression in the hippocampus because of higher sensitivity to glucocorticoids. To study the functional aspects of the hippocampus, BDNF expression and neurogenesis were also evaluated during the developmental period.

2. Materials and methods

All experiments were conducted in accordance with the guidelines set forth by the University of Tokyo in their Policies Governing the Use of Live Vertebrate Animals. These policies are based on The Public Health Service Policy on Humane Care and Use of Laboratory Animals by Awardee Institutions (rev. May 1985) and the NIH Guide for the Care and Use of Laboratory Animals (rev. 1985).

2.1. Animals

ICR mice originally obtained from Japan Clea Co., Ltd. (Yokohama, Japan) were used in all experiments. Male and female mice were pair-housed in cages (45 cm × 35 cm × 35 cm) for breeding. Food and water were given ad libitum, and the animals were kept at a constant temperature (23 ± 1 °C) and humidity (40 ± 5%) under a 12 h/12 h light–dark cycle (lights on at 08:00 h).

2.2. Weaning procedure

The weaning procedure followed that in our previous studies (Kikusui et al., 2004, 2005, 2006). Briefly, when female mice became pregnant, they were monitored daily until parturition. For each litter, the date of birth was designated postnatal day 0 (PD0). On PD2, each litter was culled to a standard size of 10 pups, with five pups of each sex. Throughout the nursing period, care was taken not to disturb the animals except for a brief weekly cage cleaning.

On PD14, half of the litter was separated from each dam and co-housed in a 12.5 cm × 20 cm × 11 cm cage. The other half of the litter was weaned at the standard age of 3 weeks (PD21). Each half of the litter consisted of two or three male pups and two or three female pups, for a total of five pups, to avoid sex segregation effects. Upon early or standard weaning, the mice were fed an ordinary adult diet (MM-3 pellets; Funabashi No-jo Co., Ltd., Chiba, Japan). On PD21, all mice were housed in single-sex groups of two or three mice of the same treatment group (early or standard weaning) in 12.5 cm × 20 cm × 11 cm cages. Each group contained two or three male or female mice from each litter to minimize inter-litter differences.

2.3. Corticosterone assay

A new group of litters was used for the corticosterone assay. On PD14, half of the litter was weaned from the dam; they were introduced to a clean bedding cage and assigned as the early-weaned group. The rest of the mice stayed with the dam. The weaning was conducted at 08:00 h and blood samples were taken by cardiac puncture from all mice in the 'weaning group' and the 'remaining with dam' group at 0 h (baseline: males $n = 9$, females $n = 9$), 0.5 h (weaned males $n = 6$, females, $n = 7$; 'remaining with dam' males $n = 4$, females $n = 4$), 1 h (weaned males $n = 6$, females $n = 5$; 'remaining with dam' males $n = 4$, females $n = 6$),