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Abnormal behavioral and neurotrophic development in the younger sibling receiving less maternal care in a communal nursing paradigm in rats

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Summary Maternal behavior in rodents has been proposed to vary as a function of the external environment and, in turn, adjust offspring's stress and fear responses. Early handling (brief periods of maternal separation during the first two weeks of life) studies and analyses of spontaneously high-caring rat mothers converge to indicate that increased levels of maternal care may reduce offspring emotionality in adulthood. However, the hypothesis that environment-dependent reduction in maternal care correlates with increased offspring vulnerability to pathology has been scarcely investigated. To test this hypothesis we studied maternal care and offspring development in young, adolescent and young-adult Sprague—Dawley rats reared in a communal nursing situation, characterized by two dams delivering their offspring four days apart and communally caring for them until weaning. We show that dams of the first-born litter show increased aggression towards the pregnant female and that offspring belonging to the second-born litter receive less maternal care compared to older cage-mates. Additionally, second-born rats show increased anxiety-related behavior in a plus-maze test in adolescence and adulthood and abnormal developmental trajectories in terms of social interaction and BDNF levels in the amygdala and hippocampus compared to both the first-born litter and to animal facility reared controls. This is the first indication that adverse environments, not requiring experimenter handling, may reduce maternal care and in turn increase offspring's emotionality and modify social behavior and BDNF developmental trajectories.

1. Introduction

Developmental plasticity of defensive responses (Macrì and Wurbel, 2006) and brain pathways (Andersen, 2003) has

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traditionally been considered the epitome of individual adaptive capacities whereby the environment shapes the individual phenotype (Laviola and Terranova, 1998; Macrì and Wurbel, 2006; Meaney, 2001). Due to its importance at many levels, identifying the nature of the interaction between an individual and its environment may (i) inform adaptive theories by describing mediators favoring individual lifetime fitness; (ii) help interpret maladaptive responses in the context of the environment (phenotypic mismatch, Bateson et al., 2004; Macrì and Wurbel, 2006; Wurbel, 2001); and (iii) refine animal models of human disorders through a directional induction of a pathologic phenotype resulting from such a mismatch.

The maternal mediation hypothesis, originally formulated by Smotherman and Bell (1980), proposed that maternal behavior in rats provides information for the growing individual. Specifically, rat mothers adjust their behavior according to environmental characteristics (e.g. food availability, predator pressure) and pups adaptively respond to match environmental requirements (Wurbel, 2001; Zhang et al., 2004). Classic and more recent studies (Levine, 1957; Levine et al., 1957; Meaney, 2001) demonstrate the dam's role in shaping phenotypic plasticity in laboratory rodents. For example, spontaneous variations in active maternal care are associated with variations in stress (hypothalamic—pituitary—adrenal, HPA) and fear responses in adult offspring (Caldji et al., 1998; Meaney, 2001). Similar findings are observed following experimenter handling manipulations (Levine, 1957; Levine et al., 1957; Macrì et al., 2004; Weininger, 1954; Weininger et al., 1954). However, two primary models have fundamental shortcomings that limit interpretation: (1) experimenter handling models involve an array of modifications (e.g. stress response in dams and pups, altered food intake) that directly affect the pup, thus hampering clear-cut mapping of environment-dependent modulation of maternal care on the offspring; although a few studies have addressed this issue (Bredy et al., 2007; Coutellier et al., in press, 2008; Macrì and Wurbel, 2007); (2) maternal separation studies (daily 3—6 h separations) increase stress and fear in the adult offspring independently of maternal care, which is increased to identical levels compared to early handling (Macrì et al., 2004; Macrì and Wurbel, 2006). Together, these limitations devalue early-life stressors as animal models of human disorders.

Here, we wanted to study whether environment-dependent reduction in maternal care, without experimenter handling, would modify offspring development in a number of behavioral responses and neurotrophic regulation (in amygdala and hippocampus) in young, adolescent, and adult rats. BDNF regulation has been proposed as a potential mediator of stress-related mood disorders (Alleva and Francia, 2009; Duman and Monteggia, 2006), and is reduced in adult offspring exposed to early maternal separation (Cirulli et al., in press; Lippmann et al., 2007). To achieve this goal, rat pups raised in a communal nursing paradigm, were born to two different dams four days apart; both dams cared for their offspring. Communal nursing in rodents allows sharing of nest duties (foraging, thermal regulation, defense against predators) due to habitat saturation of attractive niches (e.g. abundant resources) (Hayes, 2000). Communal nursing therefore may produce a model of environmental constraint, which is not found in the saturated habitat of the laboratory (unlimited food and water resources, absence of predators, constant humidity and temperature) and is not influenced by experimenter handling. We expected these conditions to elicit competition for identical resources. This procedure also allowed us to address whether maternal care by each dam was directed towards biological offspring, foster litter or both. Here, we investigated the effects of communal nursing on the developmental profile of social interaction, spontaneous locomotion and anxiety-related behavior and BDNF regulation across periods encompassing childhood, adolescence and adulthood (Andersen, 2002; Laviola et al., 1999, 2003; Paus et al., 2008; Terranova et al., 1999).

2. Methods

2.1. Animals and housing conditions

Pregnant Sprague—Dawley rats were obtained at gestation day (G)12 or G16 from Charles River Laboratories (Boston, MA). Offspring were weaned at postnatal day (P)23 and housed with same-sex littermates. Only male offspring were used from litters with <75% male:female ratio; to avoid litter effects, only one pup per cage per treatment was used in each experimental test (resulting in 6—9 rats per group per test). Rats were maintained under a 12 h light (0700—1900 h)—12 h dark cycle and free access to food and water at all times except during behavioral testing. These experiments were conducted in accordance with the 1996 Guide for the Care and Use of Laboratory Animals (NIH), and were approved by the Institutional Animal Care and Use Committee at McLean Hospital. A timeline of the experimental procedures is presented in Fig. 1. Each subject was tested only once on all behavioral paradigms adopting the following sequence: spontaneous locomotion; elevated plus maze; social interaction. This test battery was meant to respect an escalating gradient of invasiveness. Specifically we first performed the less invasive task and subsequently escalated

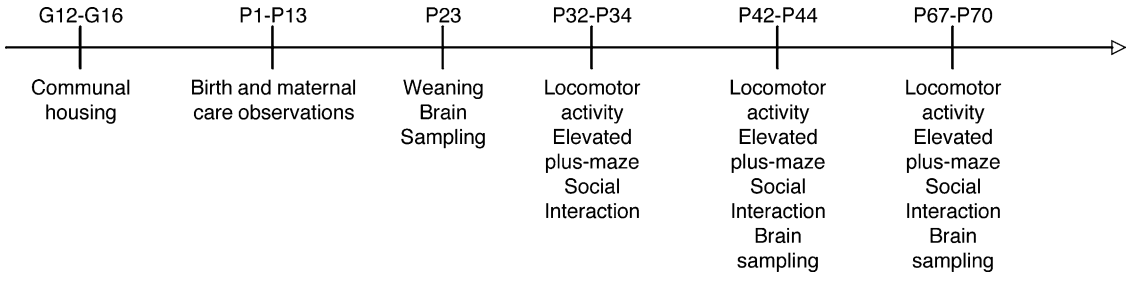

Figure 1 Experimental timeline. Synopsis of the exact timing of the experimental procedures. G—gestational day; P—postnatal day.