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Resilience and vulnerability are dose-dependently related to neonatal stressors in mice

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ABSTRACT

Early life experiences have been shown to adjust cognitive abilities, stress reactivity, fear responses and immune activity in adult mammals of many species. However, whereas severe stressors have been generally associated with the emergence of hypothalamic pituitary adreno-cortical (HPA)-mediated pathology, mild neonatal stressful experiences have been traditionally associated with 'positive' effects or resilience. External stressors stimulate the HPA axis to induce a corticosterone secretion in mouse dams, which, in turn is directly transmitted to the progeny through lactation. Such corticosteroid transfer may offer a unitary mechanism whereby early low corticosterone exposure may favor resilience in the offspring and high corticosterone increase vulnerability to pathology. In this study we further investigated this hypothesis by evaluating the long-term effects of a neonatal exposure to low (33 mg/l) and high (100 mg/l) doses of corticosterone during the first 10 days of life in outbred CD-1 mice through supplementation in the maternal drinking water. Offspring attentional set-shifting abilities, central neurotrophic regulation and levels of natural auto-antibodies (na-Abs) directed to serotonin (SERT) and dopamine (DAT) transporters were assessed in adulthood. While low levels of neonatal corticosterone improved adult cognitive abilities and increased na-Abs levels directed to SERT, high doses of neonatal corticosterone reduced hippocampal BDNF levels and na-Abs directed to DAT. These findings confirm and extend our previous findings, supporting the view that both adaptive plasticity and pathological outcomes in adulthood may depend on circulating neonatal corticosterone levels and that these effects follow a U-shaped profile.

Introduction

Mammals are adapted to occupy a wide variety of different niches. Adaptive capacities, the set of different phenotypes exhibited by a single genotype in different environments, guarantee the possibility to regulate defensive systems according to the specific requirements of the environment encountered (Bateson et al., 2004). These regulations, mainly occurring early in life (both pre- and post-natally), may result in an adaptive adult phenotype – when adjustments meet the requirements of the external environment – or in a pathologic phenotype should environmental challenges become exceedingly demanding or the adult life conditions inadequately match the perinatal adaptations (Bateson et al., 2004; Macrì and Wuerbel, 2006). It has been repeatedly demonstrated that adverse neonatal environments (e.g. abuse and neglect) may favor the onset of psychiatric disorders in adulthood (Heim and Nemeroff, 2001; Heim et al., 2008). However, just as neonatal adverse experiences may relate to the emergence of pathological phenotypes, so also a positive upbringing, matching the requirements of the future environment, may favor functional adaptations (Bateson et al., 2004).

Developmental plasticity in newborn rodents offers a key towards unravelling adaptive processes and functional and dysfunctional development (Macrì and Wuerbel, 2006). For example, adult rodents exposed to stressful neonatal environments have been shown to display behavioral, neurological and endocrine abnormalities resembling human psychiatric symptoms (e.g. Pryce et al., 2005; Ruedi-Bettschen et al., 2006; Cirulli et al., 2009). While such plasticity can be regarded as a period of vulnerability to pathology, it also serves as a window of opportunity whereby a moderately challenging environment may favor the development of an adaptive phenotype (Groothuis and Carere, 2005; Lyons and Parker, 2007; McLeod et al., 2007). Specifically, it has been demonstrated that adult rodents reared

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by dams removed from their nest for brief periods or exposed to moderately demanding foraging conditions during lactation show reduced emotionality compared to controls (Levine, 1957; Weininger, 1954; Weininger et al., 1954; Coutellier et al., 2009; Macrì et al., 2004; Macrì and Wuerbel, 2007).

Although maternal behavior has been proposed to constitute the key mediator of these adaptations (Meaney, 2001), we have shown that maternal care cannot *per se* account for such mother-offspring information transfer (Macrì and Wuerbel, 2007; Macrì et al., 2008). Specifically, we have demonstrated that variations in maternal style dissociate from adult offspring HPA regulation (Macrì and Wuerbel, 2006). We thus proposed that maternal influences on offspring phenotype might be better explained by maternal transmission of circulating levels of corticosterone through lactation, and that this form of information transfer may follow a U-shaped profile (Macrì and Wuerbel, 2006). In support of this hypothesis, D'Amato et al. (1992) have shown that brief (15-min) maternal separations are sufficient to induce a corticosterone release in both mouse dams and pups during the first two weeks of life. Whereas moderate levels of neonatal corticosterone supplemented in the maternal drinking water may reduce adult stress reactivity, HPA-associated dysfunctions, anxiety-related behavior, immune-response to pathogen infection and ischemic brain damage (Casolini et al., 2007; Catalani et al., 1993; Macrì et al., 2007), elevated levels of neonatal corticosterone may have opposite effects (Macrì et al., 2007). Additionally, developmental disruptions in the form of neonatal stress and/or exposure to elevated doses of corticosterone have been shown to deteriorate cognitive performances in the Morris water maze in rats (Choy et al., 2008).

The suitability of an hormonal information-transfer mechanism rests on several key considerations: (i) circulating levels of corticosterone vary in response to external stressors – herein considered as both positive and aversive stimuli perturbing individual homeostasis – thus constituting a valid indicator of environmental demands in terms of e.g. challenges, food availability, partner presence, predator pressure; (ii) maternal corticosterone levels are directly transmitted to the progeny through lactation; (iii) variations in maternal corticosterone levels have been shown to regulate the development of the HPA axis in the offspring; (iv) a unitary mechanism explaining both adaptive plasticity and pathological outcomes – depending on the levels of environmental demands – constitutes a parsimonious model explaining a wide spectrum of observed outcomes.

Neonatal stressors have been also associated with altered regulations of central nervous system pathways both in humans and in rodents (Duman and Monteggia, 2006; Lippmann et al., 2007). Specifically, in agreement with the observation that brain derived neurotrophic factor (BDNF) is reduced in limbic structures of depressed patients (Duman and Monteggia, 2006), maternally separated adult rats have been reported to display reduced levels of BDNF in the hippocampus (Lippmann et al., 2007). Furthermore, BDNF is known to promote the survival and differentiation of serotonergic neurons both *in vivo* and *in vitro*, thereby presumably mediating 5-HT involvement in the aetiology of emotional disturbances, (Martinowich and Lu, 2008; Martinowich et al., 2007). Finally, several diseases are associated with significant deviations in serum contents of natural auto-antibodies (na-Abs) directed to different types of brain receptors or transporter proteins. Many studies report that increased levels of na-Abs to several CNS antigens can be detected in the blood of patients with neuro-psychiatric disorders and in experimental animal models (Capone et al., 2008; Dambinova et al., 1998; Granstrem et al., 2006; Kowal et al., 2006; Vincent et al., 1999; Zandman-Goddard et al., 2007), thus suggesting that na-Abs modulations are associated with CNS (dys)functional adjustments. Here, we decided to focus on na-Abs levels to dopamine (DAT) and serotonin (SERT) transporters due to their direct involvement in the aetiology of psychiatric disorders such as depression and anxiety (e.g. Carneiro et al., 2009; Perona et al., 2008). Specifically, we hypothesized that corticosterone-induced neuro-behavioral regulations may also relate to 5-HT and dopamine brain functional adjustments and resulting na-Abs levels (Sullivan and Dufresne, 2006). Although the specific relationship between plasma na-Abs levels and target molecule function still has to be elucidated, we hypothesized that increased na-Abs related to reduced SERT and DAT function. Within this framework, we predicted an inverted U-shaped relationship between neonatal corticosterone and adult plasma na-Abs levels.

In line with our recent observations (Macrì et al., 2007), here we tested the hypothesis that the relationship between early corticosterone exposure and adult offspring regulation of cognitive abilities (attentional-set-shifting task, the rodent analogous of the Wisconsin-card-sorting task; Birrell and Brown, 2000), BDNF and na-Abs biomarkers profile follows a U-shaped curve. To test this hypothesis we analyzed the aforementioned responses in adult CD-1 mice exposed to two doses of neonatal corticosterone supplemented in the maternal drinking water during the first 10 days of life and in animal facility reared (AFR) control mice. The effects of the doses selected (30 mg/l and 100 mg/l) on levels of fluid intake, corticosterone consumption and several behavioral and immune responses have been already reported in a previous study (Macrì et al., 2007).

Methods

Subjects

Pregnant outbred CD-1 female mice (purchased from Harlan, 20050 Correzzana, MI, Italy) were housed in standard polycarbonate cages (33.0×13.0×14.0 cm) with sawdust bedding and *ad libitum* water and rodent pellets (Enriched standard diet purchased from Mucedola, Settimo Milanese, Italy). They were maintained on a reversed 12:12 h light:dark cycle (lights on at 1900 h) with temperature at 21±1 °C and relative humidity of 60±10%. Dams were inspected daily at 0930 h for delivery and day of birth was designated as postnatal day 1 (P 1). Between delivery and weaning all subjects were kept under standard Animal Facility Rearing (AFR) conditions (cage cleaning once a week). Litters were not culled until weaning and dams that delivered less than six pups or litters in which male to female ratio was heavily skewed in one or the other direction (more than 75% of same sex pups) were excluded from the experiment. Only male mice were used in the study.

At P21, offspring were weaned and re-housed in cages composed of three subjects derived from different experimental groups. Mice were marked at weaning using permanent marker. All animal handling and experimental procedures were performed according to European Communities guidelines (EC Council Directive 86/609), Italian legislation on animal experimentation (Decreto L.vo 116/92) and NIH guide for the care and use of laboratory animals.

Corticosterone administration

The day of birth was counted as P1, and, starting on P2, 7 mothers were maintained on tap water (AFR control), 7 mothers had free access to a solution of 33 μg/ml of corticosterone (L-CORT) 21-hemisuccinate (Catalani et al., 1993) and 7 mothers had free access to a solution of 100 μg/ml of CORT (H-CORT). Other than solution in the drinking bottle (water or CORT) environmental conditions were identical among groups. The treatment lasted until P10. Doses and treatment period were selected based on our previous study (Macrì et al., 2007). The doses selected resulted in a daily average corticosterone intake of 2.7±0.1 mg/mouse and of 0.9±0.03 mg/mouse in the H-CORT and in the L-CORT group, respectively. Additionally, the doses selected have been shown not to result in any alteration in fluid intake. Thus, AFR, L-CORT and H-CORT dams show similar levels of fluid intake throughout lactation (Macrì et al., 2007).