Hormones and Behavior 63 (2013) 503–509

Contents lists available at SciVerse ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Not all stressors are equal: Early social enrichment favors resilience to social but not physical stress in male mice

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ARTICLE INFO

Article history:
Received 16 October 2012
Revised 10 January 2013
Accepted 11 January 2013
Available online 23 January 2013

Keywords:
Early experiences
Communal nesting
Stress
Social
Physical
Corticosterone
Behavior
Match/mismatch hypothesis
Stress quality

ABSTRACT

Early experiences profoundly affect the adult coping response to stress and, consequently, adult vulnerability to psychopathologies triggered by stressing conditions, such as major depression. Though studies in animal models have demonstrated that individuals reared in different conditions are differently vulnerable to a stressor of a specific quality, no information is available as to whether such vulnerability differs when facing stressors of different qualities. To this purpose, we reared C57BL/6 male mice either in standard laboratory rearing condition (SN) or in Communal Nest (CN) condition, the latter consisting of a single nest where three mothers keep their pups together and share care-giving behavior until weaning. We scored the amount of interactions with the mother and with peers and found that CN is a form of social enrichment because both these components are significantly increased. At adulthood, we exposed SN and CN mice, for 4 weeks, to either a physical (forced swim) or a social stress (social instability). Immediately before, at week 1 and at week 4 of the stress procedure, corticosterone levels and the hedonic profile were measured. The results show that CN mice are more resilient to social stress than SN mice since they displayed no anhedonia and lower corticosterone levels. By contrast, both experimental groups were similarly vulnerable to physical stress. Overall, our results show that, in male mice, the adult vulnerability to stress changes according to the quality of the stressor, as a function of early experiences. In addition, the stressor to which CN mice are resilient is qualitatively similar to the stimuli they have experienced early on, both concerning the social domain.

Introduction

Chronic stress represents one of the most relevant risk factors for psychopathologies, including major depression (Kendler et al., 1995). This is supported by data showing that depression is often associated with an increase of corticotropin-releasing hormone (CRH) levels (Nemeroff et al., 1984) or, more in general, an altered function of the hypothalamic–pituitary–adrenal (HPA) axis (Holsboer, 2000; Ising et al., 2007). Furthermore, the exposure to stressful events has been reported to be one of the most powerful triggering factors for depressive episodes (Heim et al., 2009). However, the consequences of exposure to stress are not predictable just by knowing the magnitude of the stressor because vulnerability differs among individuals. For instance, while a serious life-threatening stress may not affect some individuals, a milder stress may trigger depression in others (Southwick and Charney, 2012). Recent studies have also demonstrated that individuality manifests itself according to the stressor-specific domain. In particular, different individuals show different degrees of HPA axis activation when exposed to qualitatively different stressors — *e.g.*, non-social *vs.* social stimuli (Kertes et al., 2009).

Inter-individual differences in stress response are determined by the genetic set up (Ising and Holsboer, 2006; Rijsdijk et al., 2003) and the environment in which the individual develops and lives (Taylor et al., 2004). With regard to the latter, several studies have illustrated its pervasive influence, especially during early postnatal phases, in determining the reactivity of the HPA axis at adulthood. For instance, a history of childhood abuse downregulates the HPA axis response, increasing vulnerability to stress, both at short- and long-term (Heim and Nemeroff, 2001; Lupien et al., 2009). In addition, children raised in families characterized by unsupportive relationships, emotional neglect or conflict are at increased risk of showing disruptions in stress-responsive biological regulatory systems, increasing the likelihood of developing psychiatric disorders (Repetti et al., 2002).

Animal models have been widely used to investigate the impact of the early social environment on the adult response to stress. For instance, seminal studies by Levine and others have shown that exposing rodent pups to handling, which consists in brief periods of separation from the mother for the first two post-natal weeks, reduces the HPA axis activation in response to stressful stimuli of the adult offspring (Cirulli et al., 2003; Levine, 1957; Macrì and Wurbel, 2006; Meaney, 2001; Oitzl et al., 2010; Pryce et al., 2005). These

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0018-506X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yhbeh.2013.01.003

animals also display increased exploration, reduced defecation and urination in an open field (Levine, 1957), and a reduced taste neophobia and conditioned taste aversion (Macrì et al., 2004; Weinberg et al., 1978). These studies indicate that individuals reared in different conditions are differentially vulnerable to the same stressor. However, to our knowledge, it has not been investigated whether vulnerability to stress differs when facing stressors of different qualities and whether such differential vulnerability is affected by the environment experienced during the first post-natal phase.

In the present work, we reared C57BL/6 male mice in two conditions — either the standard laboratory rearing condition (SN) or the communal nest (CN). CN consists in a single nest where three mothers keep their pups together and share care-giving behavior from birth to weaning. In this rearing condition, maternal care has been reported markedly increased (Branchi et al., 2006). Here, we scored during the first 2 weeks of life not only the amount of maternal care, but also that of peer interactions to investigate whether both these two components of early social stimulation are increased in the CN compared to the SN condition. Furthermore, as a main aim, we analyzed the coping response of SN and CN mice, in terms of adult corticosterone levels and anhedonic profile, following exposure to two qualitatively different stressors: one with a main social component and the other being predominantly physical (Fig. 1). Previous data have shown that adult CN mice display reduced anhedonia and decreased activation of the HPA axis following exposure to social stress compared to SN mice (Branchi et al., 2010; Cirulli et al., 2010). No data are available about the coping response to physical stress in CN mice and, in particular, about the coping responses to qualitatively different stressful conditions in SN and CN individuals.

Methods and materials

Animal and breeding procedure

Twenty male and 40 female C57BL/6 mice, an inbred strain were purchased from a commercial breeder (Harlan, 20050 Correzzana, MI, Italy). Upon arrival at the laboratory, the animals were housed in an air-conditioned room (temperature 21 ± 1 °C, relative humidity 60 ± 10%) with lights on from 05:00 to 17:00 h. Males and females were housed in same-sex groups of 5–6 individuals in 42 cm × 27 cm × 14 cm Plexiglas boxes with a metal top and sawdust as bedding, and with pellet food (Enriched standard diet purchased from Mucedola, Settimo Milanese, Italy) and tap water *ad libitum*. All animal handling and experimental procedures were performed according to European Communities guidelines (EC Council Directive 86/609) and Italian legislation on animal experimentation (Decreto L.vo 116/92).

Nesting condition

After 1 week of acclimatization, breeding groups (1 male and 2 females), were formed and housed in 33 cm × 13 cm × 14 cm Plexiglas boxes. Vaginal plugs were checked twice a day (at 09.00 h and 19.00 h). The male was removed on pregnancy day 12. According to the expected delivery (calculated on the basis of vaginal plug detection), mice were assigned to one of the two experimental groups: standard nesting (SN) or communal nesting (CN). For the SN group, 10 females were housed in 33 cm × 13 cm × 14 cm Plexiglas boxes.

For the CN group, 30 females were combined in trios 5 days before delivery in order to have each trio giving birth on the same day. Each trio was housed in a 42 cm × 27 cm × 14 cm Plexiglas boxes. Both in the SN and CN group, each litter was culled on the day after birth, postnatal day (PND) 1 (birth = PND 0), to six males and two females.

Pups were weaned on PND 25, and males of each litter were housed in groups of 6 animals in 42 cm × 27 cm × 14 cm Plexiglas boxes. Females were not used in this study. All behavioral studies were performed when mice were 8-month old and each behavioral test was preceded by 45–60 min of acclimatization to the experimental room. Mice were weighed at the end of the test.

Maternal behavior

Eight SN and nine CN cages were observed from PND 1 to PND 12. Maternal behavior was scored during two sessions each day. During each session, data were collected with one–zero sampling, over 30 10-s observations that were 180 s apart. The experimenter recorded whether the behavior was present or not during the 10-s observation; more than one behavior could be present and thus recorded during the same 10-s observation. The sessions started at 10:00 and 18:30. The 18:30 session was during the dark phase of the 12:12 cycle and was performed under dim red light illumination.

Maternal behavior has been analyzed according to previous work (Branchi et al., 2006). In particular, the following behaviors were scored: *arched-back nursing*: the dam is immobile and in a high upright dorsal arch posture supported by rigid fore- and hind limbs, the head is depressed, the trunk and limbs are bilaterally symmetrical, and pups are attached to the nipples; *blanket nursing*: the dam is over the pups, relatively immobile, bilaterally symmetrical, with the head not depressed, and in a low dorsal arch posture supported by rigid fore- and hind limbs or in a low dorsal arch posture supported by rigid fore limbs or rigid hind limbs or lies flat on top of the pups with little or no limb support; *passive nursing*: the dam body is lying down on her side with more than one pup usually attached to the nipples; *total nursing*: the sum of the three nursing positions; *licking/grooming*: licking and grooming of the pup body; *ano-genital licking*:

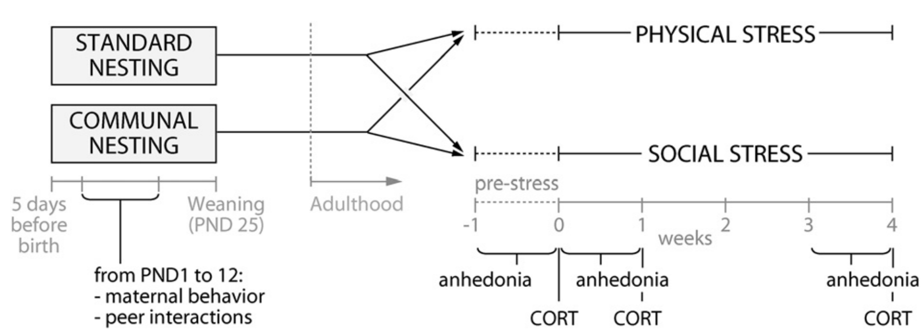

Fig. 1. Rearing conditions and experimental procedure. Scheme detailing the communal nesting manipulation and the timing of the experimental procedures. During the early post-natal phase (PNDs 1–12), the amount of maternal behavior and peer interactions have been scored. At adulthood, corticosterone levels were measured 1 day before and 1 and 4 weeks after the beginning of the chronic stress procedure. Anhedonia was assessed during the week before and the first and fourth week after the beginning of the chronic stress procedure.