Physiology & Behavior 120 (2013) 156–163

Contents lists available at ScienceDirect

Physiology & Behavior

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

Influence of housing variables on the development of stress-sensitive behaviors in the rat

Samuel A. Sakhai [a,*], John Preslik [a], Darlene D. Francis [b,c]

[a] Department of Psychology, 3210 Tolman Hall, MC 1650, University of California at Berkeley, Berkeley, CA 94720, USA
[b] Helen Wills Neuroscience Institute, 3210 Tolman Hall, MC 1650, University of California at Berkeley, Berkeley, CA 94720, USA
[c] School of Public Health, 50 University Hall, MC 7360, University of California at Berkeley, Berkeley, CA 94720, USA

HIGHLIGHTS

- Laboratory bedding material influences developmental programming of rats.
- Bedding material during early life can alter stress sensitive measures in adulthood.
- Corncob bedding during sensitive periods decreases adult measures of anxiety.
- Bedding material during adulthood does not affect adult anxiety-like behavior.

ARTICLE INFO

Article history:
Received 9 May 2013
Received in revised form 30 July 2013
Accepted 3 August 2013

Keywords:
Early-life
Housing
Bedding
Stress
Development
Rat

ABSTRACT

Diverse environments early in mammalian life can have profound influences on the physiology and behavior of developing offspring. Environmental factors can influence offspring development directly or through perturbations in parental care. In the current study, we wished to determine if the influence of a single environmental variable, type of bedding material used in laboratory cages, is capable of altering physiological and behavioral outcomes in offspring. Female rats were housed in cages containing wood pulp or corncob bedding and allowed to mature. These rats, while housed on assigned bedding material, were bred and allowed to give birth. At weaning, male offspring were housed on one of the two bedding conditions and tested later in adulthood on stress-sensitive behavioral measures. Postmortem analysis of glucocorticoid receptor expression and CRH mRNA levels were also measured. Maternal care directed at the pups reared in the two different bedding conditions was also recorded. Rats reared from birth on corncob bedding exhibited decreased anxiety-like behavior, as adults, in both open field and light–dark box tasks compared to wood pulp reared animals. Animals that received similar overall levels of maternal care, regardless of bedding condition, also differed in anxiety-like behaviors as adults, indicating that the bedding condition is capable of altering phenotype independent of maternal care. Despite observed behavioral differences in adult offspring reared in different bedding conditions, no changes in glucocorticoid receptor expression at the level of the hippocampus, frontal cortex, or corticotrophin releasing hormone (CRH) mRNA expression in the hypothalamus were observed between groups. These results highlight the importance of early life housing variables in programming stress-sensitive behaviors in adult offspring.

Published by Elsevier Inc.

1. Introduction

Across mammalian species, early life is a time of heightened susceptibility to environmental input, capable of altering the development of offspring behavior and physiology [1–4]. Environmental input, particularly during periods of heightened neuronal plasticity, can increase neuron numbers, synapses, and dendritic branching, as well as influence neuroendocrine systems such as the hypothalamic–pituitary–adrenal (HPA) or stress axis to further alter animal behavior [5–11]. In the laboratory, different animal housing conditions at various points in the lifespan of the organism (such as providing more complex/enriched cages, or conversely, deprivation) can also influence stress-axis function and behavior. Many studies, including a seminal paper by Crabbe et al. illustrate how minor changes in standard laboratory environments can abolish or reverse genetic effects of behavior in laboratory mice [12,13]. Providing enriched housing conditions to mice typically housed under standard laboratory housing conditions is sufficient enough to attenuate non-spatial memory impairments in NMDA knockout mice, possibly by increasing synaptogenesis [13,14]. Little is known about which specific features of laboratory housing contribute to changes in rodent phenotypes. One fundamental environmental variable, the type of bedding used in cages, may be a source variation in laboratory tests

* Corresponding author at: 3210 Tolman Hall, MC 1650, Berkeley, CA 94720, USA. Tel.: +1 818 635 8046; fax: +1 510 642 5293.
E-mail address: ssakhai@berkeley.edu (S.A. Sakhai).

0031-9384/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.physbeh.2013.08.003

of animal behavior. Rodent bedding materials have been demonstrated to influence stress and immune reactivity profiles [15,16], thermoregulation processes [17,18], vocalizations, [19], body mass [20], as well as liver enzyme levels in laboratory rats and mice [21,22]. Research focusing on corncob bedding, which contains measurable levels of phytoestrogens, reports alterations in slow-wave sleep, suppression of male and female reproductive behavior, acyclicity in female estrus cycles, as well as changes in estrogen receptor alpha expression in regions of the brain implicated in aggression and sexual behavior [23–25]. These studies suggest that housing conditions can fundamentally alter animal behavior and CNS function, results which emphasize the sensitivity of CNS developmental plasticity as well as fundamentally alter conclusions drawn from animal studies.

Environmental manipulations of standard laboratory housing parameters can influence offspring directly as described above, or indirectly, through perturbations in parental care. For example, alterations in early post-natal maternal care in the laboratory rat can program the developing HPA-neuroendocrine pathways and behavioral fearfulness when rodents reach adulthood [26–29]. These effects persist throughout the life of the animal and alter risk for stress-related disease [2,4,30]. Manipulation of the physical environment, including access to nesting sites and bedding, perturbs parental care which subsequently influences neuroendocrine and behavioral phenotypes of developing offspring. For example, rat mothers with restricted access to bedding material during the postpartum period displayed more disorganized/fragmented levels of maternal care than controls [31]. Rat dams themselves, with restricted access to bedding material during the postpartum period, also display an increase in HPA reactivity, more stressful behavioral phenotypes and altered hypothalamic CRH expression suggesting that environmental alterations increase maternal stress. While offspring behavior was not reported [31], other studies in which pups whose mothers were given restricted access to nesting and bedding material had deficits in spatial memory, reduced body mass, and an increase in depressive-like behavior that were accompanied by changes in hippocampal CA1 long term potentiation [5].

Using a simple manipulation of environmental parameters, we wished to investigate if the use of different housing materials during the early life period of the laboratory rat was capable of altering offspring behavior as adults and if observed changes in offspring behavior can be accounted for by alterations in maternal care. We reared Long Evans rats on wood pulp or corncob bedding, assessed maternal care during the early postpartum period and subsequently assessed stress-sensitive measures later in adulthood. We hypothesized that animals raised on wood pulp bedding conditions would differ significantly in anxiety-like behavior as adults than animals raised on the corncob bedding. We predicted that rat dams provided with wood-pulp materials would provide greater levels of maternal care to offspring which, in turn, would result in lower stress-reactivity phenotypes as adults.

2. Methods and materials

2.1. Animals and housing

Female Long Evans rats used in this study were purchased from Charles River Breeding Laboratories (Wilmington, MA). Adolescent female rats were pair housed in standard polypropylene cages (27.8 × 17.5 × 13.0 cm) containing either wood pulp or corncob bedding material (1/8″ Purelite Sanitized Corncob Bedding and Tek-Fresh Laboratory Animal Bedding, Harlan, Hayward, CA). Animals were allowed to mature for three months on the assigned bedding material. Females were then mated with male stud animals also purchased from Charles River. Male studs were housed on wood pulp bedding prior to mating. For all animals, temperature was kept constant at 20 ± 2 °C and relative humidity was maintained at 50 ± 5%. Rats were kept on a 12-h light–dark cycle (lights on 0700 h to 1900 h) and allowed access to food (Tekland Global Diet #2918) and tap water *ad libitum*. Females were allowed to give birth and maternal behavior was recorded as described below. A single 9.5 in. × 5.5 in. paper towel was provided for nesting material to all groups. At PND 21, male offspring from across litters (n = min 14/group), were weaned and pair housed in either corncob or wood pulp bedding conditions. Housing conditions at weaning were the same as that of the postpartum period. After 12 weeks of housing, animals were assessed on several stress-sensitive behavioral tasks described below. A subset of naïve animals (n = 10) housed on wood pulp bedding were switched to the opposite bedding and behaviorally tested after two weeks. Animals were euthanized and post-mortem markers assessed within 48 h of completing behavioral tasks. Breeding, weaning, and rearing of animals were performed simultaneously rather than sequentially. Housing and care of the rats were carried out in accordance with the standards and practices of the UC Berkeley Animal Care and Use Committee.

2.2. Observations of maternal behavior

Female rats were bred and permitted to give birth (n = 12). Day of birth was marked as postnatal day (PND) 0. Maternal observations were performed beginning on PND 1 and continued until PND 5 [10,11,32]. Each litter was observed for 3 h a day at the following times: 0700–0800 h, 1200–1300 h and 1900–2000 h. During each observation session, litters were observed and behaviors recorded every 1 min (*i.e.* each litter was observed 180 times per day for five days). Behaviors recorded included: mother on/off the nest and maternal licking behaviors directed at self or at pups. A distribution curve was generated by calculating the frequency with which pup-directed maternal licking was observed. Maternal licking was expressed as a percentage of the total number of observations performed for each litter. The mean frequency of maternal licking was calculated for the cohort. Animals were weaned on PND22, and pair housed with same sex littermates as described above.

2.3. Behavior

All animals were tested in two stress-sensitive behavioral tasks as adults: the Open-Field Test and the Light–Dark Box Test. Animals were tested on non-consecutive days.

2.3.1. Open-Field Test

To assess anxiety-like behavior, animals were exposed to an open field (a large circular polypropylene arena 140 cm in diameter, 61 cm in height). Each animal was placed in the open-field for 5 min and subsequent behaviors were recorded. The arena was cleaned between animals. Frequency of crosses between the outer arena (14 cm width) and the interior inner arena (112 cm diameter) and amount of time spent in the inner-arena of the open field was quantified. The behavior of each rat was recorded and analyzed by an experimenter blinded to group conditions. The greater amount of time spent in the inner arena was interpreted as a less anxious phenotype [33–35].

2.3.2. Light–Dark Box Test

Similar to the open field, the light–dark box is used to assess anxious behavior in rodents [33,35]. The light–dark box consists of two contiguous acrylic rectangular arenas (76 × 40 cm) connected by a 10 × 10 cm entrance. One arena, the dark box, is black acrylic and sheltered with a black acrylic cover while the second arena, the light box, is constructed of transparent acrylic and is open and uncovered. Animals were initially placed within the dark chamber and allowed 5 min of open exploration. The behavior of each rat was recorded and analyzed by an experimenter blind to the conditions. Time spent in the light box was quantified and interpreted as a behavioral marker of less anxious behavior [33,35].