Psychoneuroendocrinology 77 (2017) 225–235

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Paternal environmental enrichment transgenerationally alters affective behavioral and neuroendocrine phenotypes

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ARTICLE INFO

Article history:
Received 15 July 2016
Received in revised form 4 November 2016
Accepted 11 November 2016

Keywords:
Transgenerational
Environmental enrichment
HPA axis
Corticosterone
Stress response
Epigenetic inheritance

ABSTRACT

Recent studies have demonstrated that paternal stress in rodents can result in modification of offspring behavior. Environmental enrichment, which enhances cognitive stimulation and physical activity, modifies various behaviors and reduces stress responses in adult rodents. We investigated the transgenerational influence of paternal environmental enrichment on offspring behavior and physiological stress response. Adult C57BL/6J male mice (F0) were exposed to either environmental enrichment or standard housing for four weeks and then pair-mated with naïve females. The F2 generation was generated using F1 male offspring. Male and female F1 and F2 offspring were tested for anxiety using the elevated-plus maze and large open field at 8 weeks of age. Depression-related behavior was assessed using the forced-swim test. Hypothalamic-pituitary-adrenal (HPA) axis function was determined by quantification of serum corticosterone and adrenocorticotropic hormone (ACTH) levels at baseline and after forced-swim stress. Paternal environmental enrichment was associated with increased body weights of male F1 and F2 offspring. There was no significant effect on F1 offspring anxiety and depression-related behaviors. There were no changes in anxiety-related behaviors in the F2 offspring, however these mice displayed a reduced latency to immobility in the forced-swim test. Furthermore, F2 females had significantly higher serum corticosterone levels post-stress, but not ACTH. These results show that paternal environmental enrichment exerts a sex-specific transgenerational impact on the behavioral and physiological response to stress. Our findings have implications for the modelling of psychiatric disorders in rodents.

1. Introduction

Adverse life events can contribute to a multitude of psychiatric disorders including anxiety, depression and post-traumatic stress disorder (Lupien et al., 2009). It has been demonstrated that rodent models of chronic stress display elevated anxiety-like and depression-like behaviors, as well as hypothalamic-pituitary-adrenal (HPA) axis dysregulation (Russo et al., 2012). Recent studies have revealed that these effects are also present in the progeny of the affected parents. Clinical studies have shown a path for non-genetic transmission of paternal transgenerational effects including those of post-traumatic stress disorder (PTSD) to non-exposed offspring (Schick et al., 2013; Vaage et al., 2011; Yehuda et al., 2014). Genomic DNA sequence is the main carrier of biological information that is passed on to offspring; however, recent studies have demonstrated that epigenetic modifications can also be paternally transmitted to the offspring via the germ cells (Gapp et al., 2014; Rodgers et al., 2013). It is well known that adverse environments can significantly alter the epigenome and such modifications have been associated with several psychiatric disorders (Gräff and Mansuy, 2009; Ptak and Petronis, 2010; Tsankova et al., 2007). Recent work has shown that these environmentally-mediated epigenetic changes can be inherited through the paternal line and this can potentially have consequences on the inheritance of psychiatric disorders (Crews et al., 2012; Gapp et al., 2014). This emphasizes the need to identify not only genetic factors, but also

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http://dx.doi.org/10.1016/j.psyneuen.2016.11.013
0306-4530/

environmental influences on the epigenetic mechanisms that may contribute to this inheritance of predisposition to mental illness (Toth, 2014).

Recent studies have shown that stress in male mice affects the behavior of the offspring. Male offspring of stressed fathers displayed heightened anxiety, reduced sociability and increased basal levels of the stress hormone corticosterone (CORT), which also affected female offspring of the two following generations (F2 and F3) via the paternal linage (Saavedra-Rodríguez and Feig, 2013). Furthermore, maternal separation and unpredictable maternal stress (MSUS) of male mice prior to weaning altered offspring behavior for up to three generations (Franklin et al., 2010). Increased anxiety was also reported to develop in male offspring of mice which had experienced social defeat stress prior to mating (Dietz et al., 2011) and in offspring of socially isolated fathers (Pisu et al., 2013). While most studies have described the transgenerational effects of negative environments, such as stress, little is known about the effects of positive environments, such as environmental enrichment.

Environmental enrichment (EE) is an experimental paradigm that has been shown to limit behavioral, cellular and molecular pathologies in various disease models, including models of stress (Chekmareva et al., 2014; Renoir et al., 2013; Rogers et al., 2016). EE rescues the depression-like phenotype and corrects HPA-axis dysregulation in a mouse model of alcohol withdrawal (Pang et al., 2013). EE also has the capacity to influence peripheral stress-response as demonstrated by its correction of adrenal hyperresponsivity pathology in a mouse model of Huntington's disease (Du et al., 2012). In normal rodents, EE has consistently been shown to reduce anxiety-like behavior in the elevated-plus maze and the open-field test (Sztainberg et al., 2010), with recent evidence suggesting the requirement of a minimum 3-week enrichment period (Leger et al., 2015). In contrast, the anti-depressive effects of enrichment remain controversial. While there are reports that even a short 7-day period of environmental enrichment reduces forced-swim test (FST) immobility time in rats (Zanca et al., 2015), other evidence in rats and mice suggest that EE does not alter latency to immobility and total immobility time in the FST (Leger et al., 2015; Possamai et al., 2014).

Recently, it was reported that the male offspring of mice exposed to early life stress through MSUS develop a conflicting behavioral phenotype of reduced anxiety (increased time in the light half side of the light-dark box) with increased depressive behavior (increased immobility time in the FST) (Gapp et al., 2014). Interestingly, environmental enrichment prevented the transmission of paternal traumatic effects to offspring (Gapp et al., 2016). A transgenerational influence of enrichment on hippocampal function is likely to also involve enhancement of long-term potentiation properties as previously reported in a study of 2-week juvenile enrichment (Arai et al., 2009).

In this study, we have investigated the effects of 4 weeks of environmental enrichment on adult male C57Bl/6J mice to determine the transgenerational effects on offspring behavior under non-stress and non-disease conditions. As we recently showed that paternally-mediated effects on offspring anxiety could manifest across two subsequent generations (Short et al., 2016), we broadened our investigation to examination of the F2 generation.

We report that environmental enrichment had no impact on F1 offspring anxiety-related behaviors. In the F2 offspring, however, environmental enrichment did produce a quicker adaptation to floating posture in the forced-swim test of depression-like behavior. Moreover, F2 females showed significantly higher post swim-stress corticosterone levels. Interestingly, female and male F2 progeny of EE males showed elevated body weights corresponding to higher body weights of the male F1 offspring. This is the first evidence for transgenerational effects of environmental enrichment on the behavioral and physiological response to stress.

2. Material and methods

2.1. Mice

32 male C57Bl/6J mice were purchased at 7 weeks of age from the Animal Resources Centre (Murdoch, WA, Australia) and housed in the core animal facility in open-top standard laboratory mouse cages ($15 \times 30 \times 12$ cm) with *ad libitum* food and water. Mice were maintained on a 12-h light/dark cycle (lights on at 0700H). Mice were weighed once a week and the cage bedding was changed weekly. All procedures were approved by the Florey Institute of Neuroscience and Mental Health Animal Ethics Committee in accordance with the recommended guidelines set by the National Health and Medical Research Council (NHMRC) of Australia.

2.2. Environmental enrichment paradigm

At 10 weeks of age, male mice were randomly allocated into groups of 4 mice per cage to be housed under standard housing (SH) or environmentally enriched housing (EE) for 4 weeks (16 mice for each group). Enriched mice were housed in larger sized ($25 \times 38 \times 25$ cm) rat boxes with elevated lids, whereas the control mice were housed in standard mouse cages containing only bedding. Housing boxes for the EE group contained a variety of objects (such as cardboard rolls, wire, mesh, wooden and plastic objects and shredded paper), which were changed once a week in order to provide novel and complex stimulation.

2.3. Breeding

After 4 weeks of environmental enrichment, males were pair-mated with 10-week-old naïve C57Bl/6J females in standard cages for 5 days. Females were single housed until they littered down. Females who were not pregnant or lost their litter after birth were removed from the study.

On postnatal day 25, offspring were weaned and divided into new standard housing boxes. Every box comprised 3–5 mice of the same sex and same paternal housing. When offspring were 8 weeks of age, behavioral testing began for all offspring apart from 14 males from both groups (six controls and eight of paternal EE) that were used for generating the F2 generation. These males were mated at the age of 14 weeks with 10-week-old naïve females as described above. F2 offspring were weaned in the same way as F1 and were behaviorally assessed starting from 8 weeks of age.

2.4. Behavioral testing

All tests were performed during the light phase of the light/dark cycle and were completed before 1300H in order to control for time of day effects. Mice were acclimated to the room for at least 1 h before commencement of each test. From 8 weeks of age, offspring were tested on the elevated-plus maze, large open field and forced-swim test for affective behavior. The same mice were used for all the experiments. See Fig. 1A for experimental design diagram.

2.4.1. Maternal behavior

After offspring were born, the maternal behavior towards the pups was observed in two sessions each day (morning and afternoon), from postnatal day 1–5. Observations took place every 5 min for 60 min in every session and were manually scored as previously described (Chourbaji et al., 2011; Short et al., 2016).