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Research report

Parental enrichment and offspring development: Modifications to brain, behavior and the epigenome

Richelle Mychasiuk[a,*], Saif Zahir[a], Nichole Schmold[b], Slava Ilnytskyy[c], Olga Kovalchuk[c], Robbin Gibb[a]

[a] University of Lethbridge, Canadian Centre for Behavioural Neuroscience, Canada
[b] University of Calgary, Hotckiss Brain Institute, Canada
[c] University of Lethbridge, Department of Biology, Canada

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ABSTRACT

Environmental enrichment has been shown to have profound effects on the healthy adult brain and as a remedial tool for brains compromised by injury, disease, or negative experience. Based upon these findings and evidence from the prenatal stress literature, we ventured an exploratory study to examine the effects of parental enrichment on offspring development. Using Long Evans rats, paternal enrichment was achieved by housing sires in enriched environments for 28 days prior to mating with a control female. For the maternal enrichment paradigm, female rats were also housed in enriched environments for 28 days (7 days prior to conception and for the duration of pregnancy). Increased size, multiple levels for exploration, an abundance of stimulating toys, and numerous cagemates for social interaction were characteristic of the enriched environments. Offspring were assessed using two early behavioral tests and then sacrificed at postnatal day 21 (P21). Brain tissue from the frontal cortex and hippocampus was harvested for global DNA methylation analysis. Parental enrichment, preconceptionally and prenatally, altered offspring behavior on the negative geotaxis task and openfield exploratory behavior task. Paternal enrichment significantly decreased offspring brain weight at P21. Additionally, both environmental enrichment paradigms significantly decreased global methylation levels in the hippocampus and frontal cortex of male and female offspring. This study demonstrates that positive prenatal experiences; preconceptionally in fathers and prenatally in mothers, have the ability to significantly alter offspring developmental trajectories.

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1. Introduction

The mammalian brain develops and matures within an intricate network of genetic, epigenetic, and environmental influences. The plasticity of the brain allows it to adapt to individual variations in experience that will increase the likelihood of successful development. Recently it was discovered that this adaptive process begins prenatally; fetal brain development is influenced by negative maternal experiences [1–3]. Changes in offspring brain could be identified at behavioral [4], anatomical [5], and genetic [6] levels. Despite the abundance of literature illustrating the significant effects of negative maternal experience on offspring development there was very little investigation of the relationship between positive prenatal experience and offspring outcomes. The discovery by Hebb [7] that rats raised in complex environments demonstrated improved learning led to examination of enriched environments and positive experiences for remedial purposes (e.g. [8–11]), but there has been minimal examination of proactive enrichment [12,13].

In addition, current notion maintains that only maternal behaviors/experiences can influence offspring development before birth. However, recent research in humans has linked preconceptional paternal exposure to alcohol [14] and radioactive material [15], to negative offspring outcomes. Owing to the continuous nature of spermatogenesis, experiences that change methylation patterns in sperm before fertilization have the potential to alter epigenetic programming of future offspring. Past literature has demonstrated that stress alters the epigenome in rat brain [16], and more recently Potemina [17] has shown that stress impairs spermatogenesis in adult rats. As research has clearly demonstrated that environmental enrichment alters gene expression in the brain of adult rats [18], this effect should also extend to developing sperm.

The purpose of the current study was to examine the effects of parental enrichment on the brain, behavior, and epigenome of developing rat offspring. As there has been very little investigation into the effects of positive experiences on offspring development, we wanted to undertake an exploratory examination of the outcomes associated with prenatal maternal enrichment and preconceptional paternal enrichment. Two brain areas, the frontal

* Corresponding author. Tel.: +1 403 620 2549; fax: +1 403 329 2775.
E-mail address: r.mychasiuk@uleth.ca (R. Mychasiuk).

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cortex and hippocampus, were chosen for analysis because they are intricately involved in processes such as executive functioning and learning, and because they have been heavily investigated in the prenatal stress literature. In effort to detect changes in the earliest stages possible, behavioral tests were administered prior to global DNA methylation analysis in preweanling offspring. DNA methylation analysis was completed on postnatal day 21 (P21) because we were interested in determining if effects could be detected at this early stage and we wanted to avoid the confounding effects of pubertal hormones on brain methylation.

2. Materials and methods

2.1. Subjects and enrichment procedures

All experiments were carried out in accordance with the Canadian Council of Animal Care and approved by the University of Lethbridge Animal Care Committee. All animals were maintained on a 12:12 h light:dark cycle in a temperature controlled breeding room (21 °C) and were given access to food and water ad libitum. The enrichment condo was a large steel cage (2 ft × 4 ft × 6 ft) with 3 levels connected via ramps and bars. The enrichment condo contained miscellaneous "toys" that were changed on a weekly basis to continuously encourage exploration. Various foods such as peanut butter and Cheerios© were also spread around the condo to promote climbing and activity. The standard housing condition (control group) consisted of regular shoe-box cages (48 cm × 25 cm × 20 cm) and standard housing animals received the same food "treats" as offered to the enriched house animals.

2.2. Paternal enrichment

Four adult male Long Evans rats were housed in an enriched condo for 28 days and then immediately mated with a female Long Evans rat housed in a shoe-box cage. The female rat lived in a standard shoe-box cage before pregnancy, for the duration of pregnancy, and for postnatal rearing. All female dams were housed in pairs before and during pregnancy, when pups were born, females were housed individually with their litters.

2.3. Maternal enrichment

Four female Long Evans rats experienced enrichment 7 days prior to conception and for the duration of pregnancy. Female rats spent the 12 h light portion of the day in a home shoe-box cage (paired with one other female rat) and the 12 h dark portion of the day in the enrichment condo (with six female rats). This living arrangement was implemented so female dams would be accustomed to the home shoe-box cage before parturition. Female rats were habituated to the alternating living arrangement for 7 days, on the 8th day a male Long Evans rat was introduced to the condo. The male rat remained in the condo until the female dams were removed. Females were maintained in the enriched living arrangement for the duration of pregnancy. Following birth of the offspring, dams and their litters were moved permanently to standard shoe-box cages. Following birth of the pups, females were housed individually with their litters.

2.4. Control group

Male and female rats bred for control offspring lived exclusively in standard shoe-box cages. Male rats were placed with female rats for breeding and were then removed. Female dams ($n = 4$) were housed in shoe-box cages during pregnancy and postnatal rearing. When male rats were not breeding they were housed in pairs with another male rat. Female rats were housed in pairs prior to and for the duration of pregnancy. Following the birth of the pups, all females were housed individually with their litters.

2.5. Behavioral methods

2.5.1. Negative geotaxis

Pups were tested on the negative geotaxis task at P10. At this point, pups eyes are still closed. Pups were individually placed facing downward on a Plexiglas® board set to a 40° angle. Pups were filmed for 60 s. If the pup slid off the board, they were replaced in the downward direction. A pup was considered to be in a upward position when its head crossed the horizontal plane. Pups were scored for the amount of time they spent in the upward direction.

2.5.2. Open field

Pups were tested on the open field task from P10 to P13 and P15. By P15 pups eyes are beginning to open but they are closed on P10 when the task commences. Pups are individually placed in the centre of a transparent Plexiglas® box (16 cm × 20 cm × 20 cm). The base of the open field box was divided into roughly 130 squares (2 cm × 2 cm). Pups were filmed for 60 s and scored for the total number of novel squares their front paws entered. Each video was scored by two analysts blind to the treatment groups, and the average of the scores for each pup was used. As we are aware of the negative consequences associated with maternal separation [19], we wanted to limit the time pups were away from their mothers and therefore tested them for 1 min/day. This would ensure pups were only separated from their mothers for brief periods of time.

2.6. Methylation procedure

On P21, pups were subjected to isoflorane inhalation, weighed and quickly decapitated. The brains were then removed from the skull and weighed. The frontal cortex and hippocampi of each pup was removed, immersed in RNAlater® stabilization reagent (Qiagen; Valencia, California), immediately flash frozen on dry ice, and stored at −80 °C. DNA was extracted from the tissue using the Allprep DNA/RNA mini kit [20].

To determine the extent of global methylation, a well-established radiolabeled [3H]-dCTP HpaII/MspI-based cytosine extension assay was used [21]. This assay measures the proportion of CpG islands that have lost methyl groups on both strands of the DNA. In brief, 1 μg of genomic DNA is digested with the methylation-sensitive HpaII restriction endonuclease (New England Biolabs; Beverly, MA) while a second 1 μg of genomic DNA is digested with the methylation-insensitive endonuclease MspI (New England Biolabs; Beverly, MA). A final 1 μg of genomic DNA is left undigested to serve as a background control. The single nucleotide extension reaction is performed in a 25 μl reaction mixture containing 1 μg DNA, 1 X PCR buffer II, 1.0 mM $MgCl_2$, .25 U AmpliTaq DNA polymerase, and 1.0 μl of [3H]-dCTP, that is incubated at 56 °C for 1 h. Samples were then applied to DE-81 ion-exchange filter paper and washed three times with .5 M Na-phosphate buffer (pH 7.0). The filter paper is then dried and processed for scintillation counting. Two technical repeats of each experiment were conducted to ensure consistency of the data. The absolute percentage of double-stranded unmethylated CpG sites can be calculated by relating the data of HpaII and MspI digests [22].

2.7. Statistical analysis

All statistical analysis was carried out using SPSS 16.0 for Mac. Analysis was conducted to ensure results could not be attributed to a single litter or single parent. Two-way ANOVA's with Enrichment and Sex as factors were run to compare the paternal and maternal enrichment offspring to control offspring. Significant results in all graphical representation are reported as significant differences between either of the enrichment groups and the no-enrichment control group.

3. Results

3.1. Parental and litter characteristics

There was no effect of enrichment on length of pregnancy or male to female ratio of offspring. Sires in the shoe-box cages gained significantly more weight over the 4 week enrichment period prior to conception than sires living in the condos (weight gain during paternal enrichment; (grams): control, 54.75 ± 15.23; paternal enrichment, −22.00 ± 12.30; $F(1,7) = 12.85$, $p = .01$). Weight gain during pregnancy did not significantly differ for dams in the maternal enrichment group when compared to control dams, $p > .05$.

3.2. Brain weight

Brain weight was computed as a percentage of body weight. Both male and female offspring of prenatally enriched fathers exhibited a decrease in brain weight when compared to control offspring. Conversely, maternal enrichment had no effect on brain weight. A two-way ANOVA with enrichment and sex as factors showed a main effect of enrichment, $F(2, 99) = 6.76$, $p = .002$, but not of sex, $F(1, 99) = 1.56$, $p = .22$. The interaction was not significant, $p > .05$. See Fig. 1.

3.3. Negative geotaxis

Both male and female offspring of enriched mothers exhibited a reduction in time spent in the upward direction when compared to control offspring. There was no change in performance on the negative geotaxis task for offspring of enriched fathers. A two-way ANOVA with enrichment and sex as factors showed a main effect

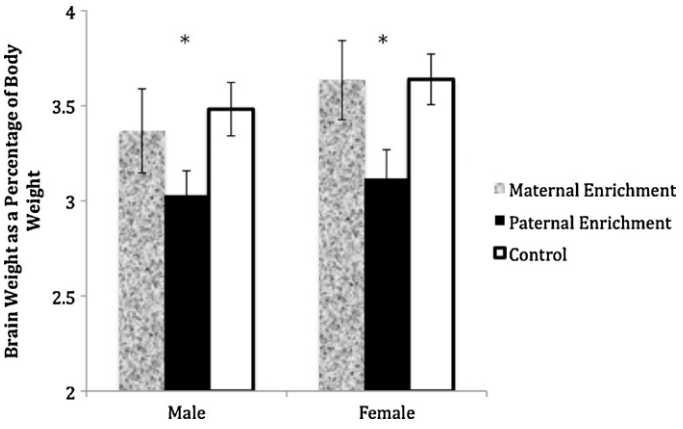

Fig. 1. Average brain weight as percentage of body weight for offspring sacrificed at P21. Significant results are based on comparisons made between control offspring and enriched offspring, $p < .05$.

of enrichment, $F(2, 103) = 2.97$, $p = .05$, but not of sex, $F(1, 103) = .20$, $p = .66$. The interaction was not significant, $p > .05$. See Fig. 2.

3.4. Open field

When total number of novel fields entered is summed across all 5 days: activity level for both male and female offspring in the maternal enrichment group is increased, along with activity level for male offspring in the paternal enrichment group. A two-way ANOVA with enrichment and sex as factors showed a main effect of enrichment, $F(2, 103) = 8.36$, $p < .01$, but not of sex, $F(1, 103) = .00$, $p = .98$. The interaction was not significant, $p > .05$. See Fig. 3.

3.5. Global DNA methylation

3.5.1. Hippocampus

A decrease in global DNA methylation was seen in both male and female offspring of the maternal and paternal enrichment groups when compared to control offspring. A two-way ANOVA with enrichment and sex as factors demonstrated a main effect of enrichment, $F(2, 53) = 3.88$, $p = .03$, but not of sex, $F(1, 53) = .08$, $p = .78$. The interaction was not significant, $p > .05$. See Fig. 4.

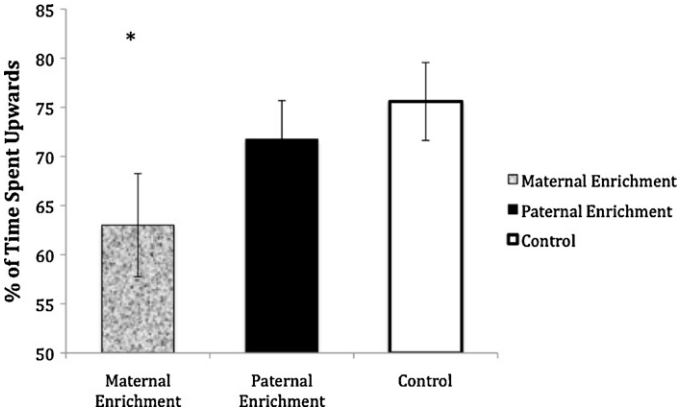

Fig. 2. Average time offspring (P10) spent in the upwards direction on the negative geotaxis task. Significant results are based on comparisons made between control offspring and enriched offspring, $p < .05$. As there were no significant effects of sex, data were collapsed across sexes.

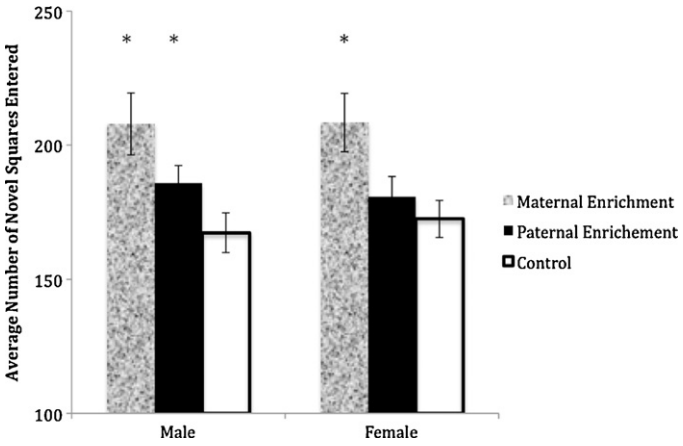

Fig. 3. Average number of novel squares offspring entered summed for all 5 testing days (P10-P13 and P15). Significant results are based on comparisons made between control offspring and enriched offspring, $p < .05$.

3.6. Frontal cortex

Similarly, both male and female offspring in both of the enrichment groups exhibited a significant decrease in global DNA methylation in the frontal cortex when compared to control offspring. A two-way ANOVA with enrichment and sex as factors showed a main effect of enrichment, $F(2, 51) = 3.45$, $p = .04$, but not of sex, $F(1, 51) = .01$, $p = .94$. The interaction was not significant, $p > .05$. See Fig. 4.

4. Discussion

There is a plethora of research demonstrating the effects of negative prenatal experiences on brain and behavioral development (e.g. [1,3,6,23,24], but there has been very limited examination of outcomes associated with positive prenatal experiences [12,25,26]. Irrespective of the pattern of behavioral and epigenetic outcome, this study has clearly demonstrated that pre-conception and prenatal enrichment experiences can significantly alter offspring developmental trajectories.

4.1. Effects of enrichment on brain weight

Prenatal maternal enrichment and pre-conceptional paternal enrichment differentially affected offspring brain weight. Prenatal maternal enrichment did not change offspring brain weight, where

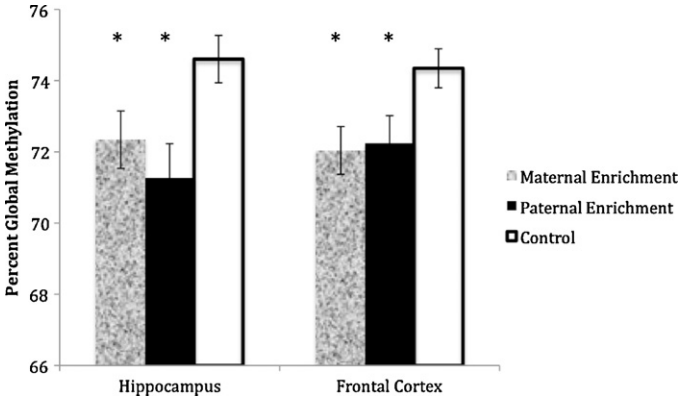

Fig. 4. Average global DNA methylation levels in the hippocampus and frontal cortex of offspring at time of sacrifice (P21). Significant results are based on comparisons made between control offspring and enriched offspring, $p < .05$. As there were no significant effects of sex, data were collapsed across sexes.

as both male and female offspring in the paternal pre-conception enrichment group exhibited reduced brain weight when compared to control offspring. The reduction in brain weight of males in the paternally enriched group is similar to results from Muhammad and Kolb (2011) [12] who found reduced brain weight in male offspring born to mothers undergoing tactile stimulation during pregnancy. It is possible that the reduced brain weight is associated with more efficient synaptic connectivity in these offspring. Owing to evidence identifying increased dendritic branching and spine density in the brains of enriched adults [27], the lack of change in maternally enriched offspring brain weight was surprising. However, this may have been a result of the early age at which the offspring were sacrificed; these offspring may exhibit changes in brain weight in adulthood.

4.2. Effects of enrichment on behavior

Offspring of enriched rats were tested on two different tasks to investigate the effects of parental enrichment on early behavior. Both male and female offspring in the maternal enrichment group exhibited a reduction in the amount of time spent upwards on the negative geotaxis platform. Although the skills needed to complete this task are not fully understood, it is often used as a milestone for sensorimotor development in young rats [28,29]. It is possible that offspring in this group demonstrate decreased performance on the task because maternal enrichment during pregnancy is slowing brain maturation of offspring, which should promote greater plasticity later in life. However, as there has been little research conducted on positive prenatal experiences this is only speculation. Offspring in the paternal enrichment group performed equally as well as control offspring on the negative geotaxis task.

Offspring behavior in the open field was significantly altered in response to parental enrichment. Both male and female offspring in the maternal enrichment group exhibited increases in exploratory behavior, where as only male offspring in the paternal enrichment group demonstrated an increase in exploration. This is similar to results obtained by Dell and Rose (1987) [25], who found increases in female offspring activity following maternal enrichment. Contradictorily, however, they did not report significant differences in activity of male offspring. This discrepancy in outcome may have resulted from differences in offspring age at testing. Our open-field testing occurred from P10 to P15 (preweaning) whereas Dell and Rose (1987) [25] tested from P22 to P26 (postweaning).

4.3. Enrichment induces epigenetic change

Both male and female offspring exposed to parental enrichment exhibit significant reductions in global DNA methylation in the hippocampus and frontal cortex. The substantial reduction in DNA methylation implies an increase in gene expression across enrichment groups and sexes. This finding is consistent with gene expression research in enriched adult rats [18]. Similarly, the decreased levels of DNA methylation and implied increase in gene expression in the hippocampus may help explain discoveries of increased neurogenesis [30] and improved learning [31,32] in rats living in enriched environments. It could also be hypothesized that the changes in frontal cortex methylation may be related to the protective role positive experiences play in brain development (For review see, [33].

Based on fetal programming theories [34], alterations to the genome of offspring in the maternally enriched group were expected. Surprisingly, and most interestingly, enriching fathers preconceptionally, significantly altered global methylation levels in the brains of developing offspring. As sperm development in Long-Evans rats occurs continuously, just as in humans, it is likely that enrichment altered gene expression in the sperm of sires, providing a means for the transmission of epigenetic change to the offspring. Regardless of the mechanism, this finding has major implications for “soon to be fathers”.

5. Conclusion

Although the exact nature of the gene–stress interaction has not been established, the transmission of glucocorticoids from mother to offspring has been identified as an underlying mechanism responsible for the relationship between prenatal stress and offspring outcome. Owing to limited research on positive early experiences, we are unable to provide a mechanistic theory that would explain how parental enrichment (preconceptually or prenatally) induces genome wide methylation changes in offspring brain, especially specific to the hippocampus or frontal cortex. In addition, we recognize that in using our DNA methylation technique we are unable to differentiate between methylation of gene promoter regions and methylation of transposable elements. We are aware that transposable elements are also highly susceptible to epigenetic modification [35]. However, because research has indicated that changes in methylation of transposable elements is also related to gene activity [36], and this study was designed to be an exploratory analysis of epigenetic modifications in the brain, this is not a major limitation. Hence, we have identified an interesting and important phenomenon that has large implications for human development and therefore requires further investigation.

References

[1] Kapoor A, Dunn E, Kostaki A, Andrews MH, Matthews S. Fetal programming of hypothalamo-pituitary-adrenal function: prenatal stress and glucocorticoids. J Physiol 2006;572:31–44.
[2] Kawamura T, Chen J, Takahashi T, Ichitani Y, Nakahara D. Prenatal stress suppresses cell proliferation in the early developing brain. Dev Neurosci 2006;17:1515–8.
[3] Lemaire V, Koehl M, Le Moal M, Abrous D. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc Natl Acad Sci 2000;97:11032–7.
[4] Lee P, Brady D, Shapiro R, Dorsa D, Koenig J. Prenatal stress generates deficitis in rat social behavior: reversal by oxytocin. Brain Res 2007;1156:152–267.
[5] Martinez-Tellez R, Hernandez-Torres E, Gamboa C, Flores G. Prenatal stress alters spine density and dendritic length of nucleus accumbens and hippocampus neurons in rat offspring. Synapse 2009;63:794–804.
[6] Mychasiuk R, Ilnystkyy S, Kovalchuk O, Kolb B, Gibb R. Intensity matters: brain, behaviour, and the epigenome of prenatally stressed rats. Neuroscience 2011;180:105–10.
[7] Hebb DO. The effects of early experience on problem solving at maturity. Am Psychol 1947;2:306–7.
[8] Yang J, Hou C, Ma N, Liu J, Zhang Y, et al. Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress. Neurobiol Learn Mem 2007;87:257–63.
[9] Morely-Fletcher S, Rea M, Maccari S, Laviola G. Environmental enrichment during adolescence reverses the effects if prenatal stress on play behavior and HPA axis reactivity. Eur J Neurosci 2003;18:3367–74.
[10] Solinas M, Chauvet C, Thiriet N, El Rawas R, Jaber M. Reversal of cocaine addiction by environmental enrichment. Proc Natl Acad Sci 2008;105:17145–50.
[11] Laviola G, Hannan A, Macri S, Solinas M, Jaber M. Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disordes. Neurobiol Dis 2008;31:159–68.
[12] Muhammad A, Kolb B. Prenatal tactile stimulation attenuates drug-induced behavioral sensitization, modifies behavior, and alters brain architecture. Brain Res 2011;1400:53–65.
[13] Schneider JS, Lee MH, Anderson DW, Zuck L, Lidsky TI. Enriched environment during development is protective against lead-induced neurotoxicity. Brain Res 2001;896:48–55.
[14] Abel E. Paternal contribution to fetal alcohol syndrome. Addict Biol 2004;9:127–33.
[15] Miller AC, Stewart M, Rivas R. Preconceptional paternal exposure to depleted uranium: transmission of genetic damage to offspring. Health Phys 2010;99:371–9.
[16] Kitraki E, Karandrea D, Kittas C. Long-lasting effects of stress on glucocorticoid receptor gene expression in the rat brain. Neuroendocrinology 1999;69:331–8.
[17] Potemina TE. Impairment of spermatogenesis in male rats during stress. Bull Exp Biol Med 2008;145:700–2.
[18] Rampon C, Jiang C, Dong H, Tang Y, Lockhart D, et al. Effects of environmental enrichment on gene expression in the brain. Proc Natl Acad Sci 2000;97:12880–4.

[19] Monroy E, Hernandez-Torres E, Flores G. Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. J Chem Neuroanat 2010;40:93–101.
[20] Qiagen (2005) AllPrep DNA/RNA mini handbook. Valencia, CA.
[21] Pogribny I, Yi P, James SJ. A sensitive new method for rapid detection of abnormal methylation patterns in global DNA and within CpG islands. Biochem Biophys Res Commun 1999;262:624–8.
[22] Koturbash I, Boyko A, Rodriguez-Juarez R, McDonald J, Tryndyak V, et al. Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. Carcinogenesis 2007;28:1831–8.
[23] Murmu M, Salomon S, Biala Y, Weinstock M, Braun K, et al. Changes in spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. Eur J Neurosci 2006;24:1477–87.
[24] Macri S, Granstrem O, Shumilina M, Gomes dos Santos F, Berry A, et al. Resilience and vulnerability are dose-dependently related to neonatal stressors in mice. Horm Behav 2009;56:391–8.
[25] Dell PA, Rose FD. Transfer of effects from environmentally enriched and impoverished female rats to future offspring. Pysiol Behav 1987;39:187–90.
[26] Laviola G, Rea M, Morely-Fletcher S, Di Carlo S, Bacosi A, et al. Beneficial effects of enriched environment on adolescent rats from stressed pregnancies. Eur J Neurosci 2004;20:1655–64.
[27] Leggio MG, Mandolesi L, Federico F, Spirito F, Ricci B, et al. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in rat. Behav Brain Res 2005;163:78–90.
[28] Alberts JR, Motz BA, Schank JC. Positive geotaxis in infant rats (*Rattus norvegicus*): a natural behavior and a historical correction. J Comp Psychol 2004;118:123–32.
[29] Patin V, Lordi B, Caston J. Does prenatal stress affect the motoric development of rat pups? Brain Res Dev Brain Res 2004;149:85–92.
[30] Kempermann G, Gast D, Gage F. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002;52:135–43.
[31] Arai J, Feig L. Long-lasting and transgenerational effects of an environmental enrichment on memory formation. Brain Res Bull 2011;85:30–5.
[32] Kiyono S, Seo M, Shibagaki M, Inouye M. Facilitative effects of maternal environmental enrichment on maze learning in rat offspring. Physiol Behav 1985;34:431–5.
[33] Nithianantharajah J, Hannan A. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci 2006;7:697–709.
[34] Talge N, Neal C, Glover V. Early stress translational research and prevention science network antenatal maternal stress and long-term effects on child neurodevelopment: how and why? J Child Psychol Psychiatry 2007;48:245–61.
[35] Das R, Hampton D, Jirtle R. Imprinting evolution and human health. Mamm Genome 2009;20:563–72.
[36] Rakyan V, Blewitt M, Druker R, Preis J, Whitelaw E. Metastable epialleles in mammals. Trends Genet 2002;18:348–51.