



## Research report

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

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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

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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 an 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 isoflurane 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<sub>2</sub>, .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,  $54.75 \pm 15.23$ ; paternal enrichment,  $-22.00 \pm 12.30$ ;  $F(1,7) = 12.85$ ,  $p = .01$ ). 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

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