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Altered gene-expression profile in rat plasma and promoted body and brain development by environmental enrichment

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Environmental enrichment (EE) refers to the exposure of laboratory animals to physical and social stimulation, which can improve animals' well-being. The study was aimed to explore how the prenatal EE impacts affect the development, behavior, hormones and gene expression of the offspring. 28 pregnant rats were randomized into an EE group (EEG) housed in cages with EE or a control group (CG) housed in normal cages. Measurements included offspring development parameters (body weight, body length, and tail length) and behavior (open-field test, OFT), hormone levels (cortisol, dopamine, 5-HT, and growth hormone) and gene expression profile. Results showed that the development parameters of EEG offspring were statistically superior to the CG offspring. OFT count of EEG offspring was more than CG. EEG and CG offspring did not differ on cortisol, dopamine, 5-HT or growth factor. Gene expression profile chip test showed that 25 genes were up-regulated and 23 genes down-regulated in the EEG vs CG comparison, among which five GO annotations and four KEGG pathways were annotated. Findings indicate that EE during pregnancy could positively promote the body and nervous system development of offspring, involving the evidence for altered gene expression profile.

Key words: Environmental enrichment, rats, gene expression, behavior, development.

INTRODUCTION

Environmental enrichment (EE) refers to the exposure of laboratory animals to physical and/or social stimulation which is superior to standard housing conditions (Zhang et al., 2012). EE has become a growing practice in animal husbandry, with its positive effect on animal psychology as well as physiology documented (Toth et al., 2011). Neuroscience has seen a wide scope of researches on EE showing benefits in several neurodegenerative and psychiatric disorders, including sleep disorders, depression, anxiety, drug addiction, Alzheimer's and Parkinson's disease, and traumatic brain injury (Simpson et al., 2011; Hill et al., 2007). Maternal effects have been demonstrated as an essential factor for offspring development in many species. The long-term perinatal mother-infant interaction leaves a strong impact upon offspring development and variation, including both psychological and physiological health (Simpson and Kelly, 2011). Mychasiuk et al. (2012) reported that

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Abbreviations: EE, Environmental Enrichment; **CG,** control group; **EEG,** environmental enrichment group; **OFT,** open-field test.

parental EE, preconceptionally and prenatally, altered offspring's behavior on the negative geotaxis task and open-field exploratory behavior task.

The maternal care an infant receives in early life can alter the development of neural systems resulting in alterations in the response to novelty and in social behavior (Cancedda et al., 2004). In short, substantial research indicates that prenatal EE alters the development and behavior of offspring, with long-term impact on psychological and physiological health. Even though there in no report on how prenatal EE affect the body development of the offspring, it is reasonable that prenatal EE may generate a promotion on the body development of the offspring. As one example of these long-term effects, a growing body of researches has examined how the changes consequent to EE can influence genes. Rampon et al. (2000) reported a large number of genes changes expression in response to enrichment training, many of which are linked to neuronal structure, synaptic plasticity and transmission. A number of these genes may play important roles in modulating learning and memory capacity.

Although, a large amount of researches have focused on the enriched-environmental genetic modifications of mammal animal models; in the same generation the development and genetic changes of the offspring resulting from the maternal EE remains to be unknown.

MATERIALS AND METHODS

Grouping

30 Sprague-Dawley (SD) female rats (230 ~ 270 g) and 30 male rats (225 ~ 261 g) were involved in this research. The rats were housed in a room at the temperature of 22°C, with a 12 h light/dark cycle and were fed with food and water *ad libitum*. After a week of acclimation, the female rats were mated with the male rats. Pregnancy was confirmed by vaginal plug test. The 23 pregnant rats were randomized into two groups; environmental enrichment group (EEG) (n = 12) and control group (standard rearing; CG) (n = 11). The two housing conditions were sustained until delivery. The two groups did not differ significantly on gestation time and body weight of the first day of gestation [EEG: 235.21 ± 1.59, CG: 234.87 ± 2.20, t test, $p>0.05$ (g)]. After delivery, all the litters of both groups were housed with their mothers in a standard pregnant rat rearing cage, one housing case one nest, until the 25th day after birth.

Housing

CG consisted of a standard pregnant rat rearing cage, with 5 to 6 rats per cage. EEG consisted of a larger cage (100 × 100 × 100 cm) with 12 rats per cage, with different objects (shelter, ladder, ball, stair, and tunnel), chews (nuts, toys), nesting material, and fetus education music twice a day in the morning and night.

Growth measurement

Body weight (g), body length (cm), and tail length (cm) were measured on the day of delivery (day 0), as well as 5 , 10 , 15 , 20 and 25 days after delivery in order to evaluate the body development of the offspring.

Open field test (OFT)

The 25 days old was placed in the center of a square board (90 × 90 cm) painted with yellow and white squares (15 × 15 cm). The number of squares the offspring had crawled across in 2 min was counted (offspring were given points only if all four claws were in a given square).

Hormones determination

Ten offspring were randomly selected from each group, and a blood sample was taken from *Arteria femoralis*. ELISA (R&D Systems China Co., Ltd.) was employed to determine the serum level of cortisol (DZE 30590), dopamine (Kit DZE 30238), 5-HT (DZE 30326) and growth hormone (DZE 30549).

Gene expression profile chip experiments

RNA extraction and purification

Total RNA was extracted using TRIZOL Reagent (Cat #15596-018, Life technologies, Carlsbad, CA, US) under the manufacturer's instructions and was checked for a RIN number to inspect RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Qualified total RNA was further purified by RNeasy mini kit (Cat#74106, QIAGEN, GmBH, Germany) and RNeasy micro kit (Cat#74004, QIAGEN, GmBH, Germany) and RNase-Free DNase Set (Cat#79254, QIAGEN, GmBH, Germany) (Table 1).

RNA amplification and labeling

Total RNA was amplified and labeled by 'low input quick amp labeling kit, one-color' (Cat # 5190-2305, Agilent technologies, Santa Clara, CA, US) under the manufacturer's instructions. Labeled cRNA were purified by RNeasy mini kit (Cat#74106, QIAGEN, GmBH, Germany).

Hybridization

Each slide was hybridized with 1.65 µg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Cat#5188-5242, Agilent technologies, Santa Clara, CA, US) in Hybridization Oven (Cat#G2545A, Agilent technologies, Santa Clara, CA, US), under the manufacturer's instructions. After 17 h hybridization, slides were washed in staining dishes (Cat#121, Thermo Shandon, Waltham, MA, US) with Gene Expression Wash Buffer Kit (Cat#5188-5327, Agilent technologies, Santa Clara, CA, US), followed by the manufacturer's instructions.

Data acquisition

Slides were scanned by Agilent Microarray Scanner (Cat#G2565CA, Agilent technologies, Santa Clara, CA, US) with default settings; Dye channel: Green, Scan resolution = 5 µm, PMT 100%, 10%, 16 bit. Feature extraction software 10.7 (Agilent technologies, Santa Clara, CA, US) raw data were normalized by Quantile algorithm, Gene Spring Software 11.0 (Agilent technologies, Santa Clara, CA, US) (Table 1).

Table 1. QC of RNA extraction and slides experiment (A sample is qualified only when 2100 RIN ⩾ 7.0 and 28S/18S >= 0.7) (1_2~1_4refers to EEG and 4_1~4_3 refers to CG).

Sample	QC of RNA							QC of Slides	
	Concentration (μg/μl)	Volume (μl)	Total (μg)	A260/A280	2100 Result		Result	CV (%)*	Detection rate (%)
					RIN	28S/18S			
1_2	0.700	50	34.98	1.93	9.0	1.4	Qualified	6.18	54.19
1_3	0.171	50	8.57	1.92	9.2	1.6	Qualified	5.90	59.87
1_4	0.168	50	8.41	1.88	9.4	1.7	Qualified	4.98	67.57
4_1	0.185	50	9.27	1.91	9.4	1.7	Qualified	6.33	61.09
4_2	0.595	50	29.75	1.93	9.4	1.7	Qualified	5.70	70.68
4_3	0.355	25	8.87	1.85	9.3	1.6	Qualified	4.39	65.52

*: CV = SD/Mean ×100.

Real-time PCR

Primers of the six genes were designed with primer express 2.0 (Oebiotec, Shanghai, China) (Table 2). Reverse transcription was performed on PrimerScript RT reagent Kit [TaKaRa, DRR037A, Takara Biotechnology (Dalian) Co., Ltd. China]. Total RNA (0.5 μg) was denatured at room temperature and then mixed with the reagent in a final volume of 10 μl containing 50 μM oligo dT, 100 μM random primer, 0.5 mM dNTP and the manufacturer's buffer and 'enzyme mix'. The RT reaction was carried out for 15 min at 37 and 85°C for 5 s in ABI 9700. First-strand cDNA product was diluted in 100 μl distilled water and ready for real-time PCR. qPCR was performed using SuperReal PreMix (SYBR Green) kit [TIANGEN, FP204, Tiangen Biotech (Beijing) Co., Ltd. Beijing, China]. Briefly, 1 μl of diluted cDNA product was utilized for 40-cycle three-step PCR in a Roche HOLD CYCLE LightCycler 480 II.

Statistical analysis

The body development, behavioral test, and hormone level data were analyzed using a statistical package for the social sciences (SPSS) version 19.0 with alpha set to 0.05. Repeated measures ANOVA with Greenhouse-Geisser adjustment was performed to analyze the difference of body weight, body length and tail length across days. A nonparametric test was performed to analyze the difference of OFT. Student's t-test was performed to analyses the difference in the levels of cortisol, dopamine, 5-HT and growth hormone.

RESULTS

Alteration of body development

Repeated measured ANOVA with Greenhouse-Geisser adjustment (Table 3) showed the body weight, body length and tail lengths of EEG offspring were statistically superior to those of the CG offspring (Table 4 and Figure 1). Table 5 shows the comparison detail of the six observation times (day 0, 5, 10, 15, 20 and 25).

Behavior tests

A nonparametric test showed significant difference between EEG and CG that is, EG showed more cells than CG (Mann-Whitney U = 1448.500, Wilcoxon W = 2529.500, Z = -3.819, p = 0.000) (Figure 2).

Hormones tests with ELISA

EEG and CG did not differ significantly on serum levels of cortisol, dopamine, 5-HT or growth hormone (all p's >0.05). However, the growth hormone level of EEG were non significantly higher than CG (p = 0.088) (Table 6).

Gene expression profile

Gene expression profile allowed for a comparison of 48 genes between groups. EEG and CG groups differed significantly: 25 genes were up-regulated and 23 genes were down-regulated (p<0.01) in EEG vs CG (Table 7, Figure 3), among which five GO annotations were obtained including nitrogen compound metabolic process, thioredoxin-disulfide reductase activity, catabolic process, phosphate binding, and hydrolase activity (Table 8), and among which four KEGG pathways were annotated, including circadian rhythm, ether lipid metabolism, endocytosis and primary immunodeficiency (Table 9).

Table 2. Primers and product lengths of the six targeted genes.

Number	Gene Symbol	Forward Primer	Reverse Primer	Product Length
1	*ACTB	GCGTCCACCCGCGAGTACAA	ACATGCCGGAGCCGTTGTCG	118
2	Irf7	TGGCAGATGGAAGCTACC	GGCTATACAGGAACACGC	154
3	Ninj2	CCACCACCTTGGTCTTCATA	AGGCTGAAGTGGCTTTAG	152
4	Bhlhe41	CTTGTGATGGGAAGTGCT	ATCTAGACAAGGTCTCGGT	152
5	Kynu	CGGATGATAAAGCCACGAG	GTGTAATGGCAGGAATGTTG	151
6	Blnk	CTGGGACTCCAAGTCAT	ACAGTGTCTTGTCTGTGACTA	180
7	Pde6h	TCGGGATCATCTGAGGAC	GAAGGAAAGAACACGACGG	159

* Refers to internal control gene.

Table 3. Mauchly's Test of sphericity for the body weight, body length, and tail length.

Within subjects effect	Mauchly's W	Approximate Chi-Square	df	Significant	Greenhouse-Geisser
Body Weight	0.024	531.364	14	0.000	0.416
Body Length	0.621	67.718	14	0.000	0.848
Tail Length	0.409	126.320	14	0.000	0.735

Table 4. Tests of Within-Subjects Effects for the body weight, body length and tail length.

Source	df	Mean Square	F	Significance
Body_Weight	3.705	564.763	4127.877	0.000
Body_Weight* Group	11.115	4.612	33.709	0.000
Body_Length	4.241	733.953	2837.266	0.000
Body_Length* Group	4.241	3.538	13.678	0.000
Tail_Length	3.675	485.717	3654.380	0.000
Tail_Length* Group	3.675	2.835	21.327	0.000

RT-PCR validation

Six genes (Irf7, Ninj2, Bhlhe41, Kynu, Blnk, Pde6h) were filtered to validate with RT-PCR according to the set, the flag value of the expression profile chip ≠A, FC>2 or FC<0.5, expression value ≥ 6 from the GO and KEGG annotation. As shown in Figure 4, Irf7 and Ninj2 were hyper-expressed in EEG, while Bhlhe41, Kynu, Blnk and Pde6h were hypo-expressed. The gene expression profile chip outcome was perfectly in accordance with the RT-PCR result.

DISCUSSION

Experimental EE, as applied in adulthood or immediately after weaning has been shown to have robust impact on behaviors and physiology. Findings of this study demonstrate that prenatal EE could significantly improve offspring development including body weight, body length, and tail length, which may be correlated to the increased hormone level in EEG. Although, relatively few studies have examined maternal EE, multiple studies have indicated that prenatal stress may induce intra-uterine growth retardation (Drago et al., 1999). Findings showed that EE improved OFT performance. Previous studies reported that EE could increase exploratory behavior (Mychasiuk et al., 2012; Maruoka et al., 2009). For instance, Mychasiuk et al. (2012) found that maternal EE significantly affected the locomotor activity in female rats. Serum cortisol, dopamine, and growth hormone levels of EEG did not differ from the control condition. The result might be limited because serum samples were taken to determine the hormone levels rather than brain structures. There is controversy on whether EE produces changes in serotonin or DA level, with mixed findings across studies (Simpson and Kelly, 2011).

In one of the few studies of prenatal EE and hormones, Welberg et al. (2006) found no significant effect of maternal EE on basal ACTH and CORT levels in adult male offspring. In gene expression profile compared with CG, the outcome of EEG was characterized by 25 up- regulated

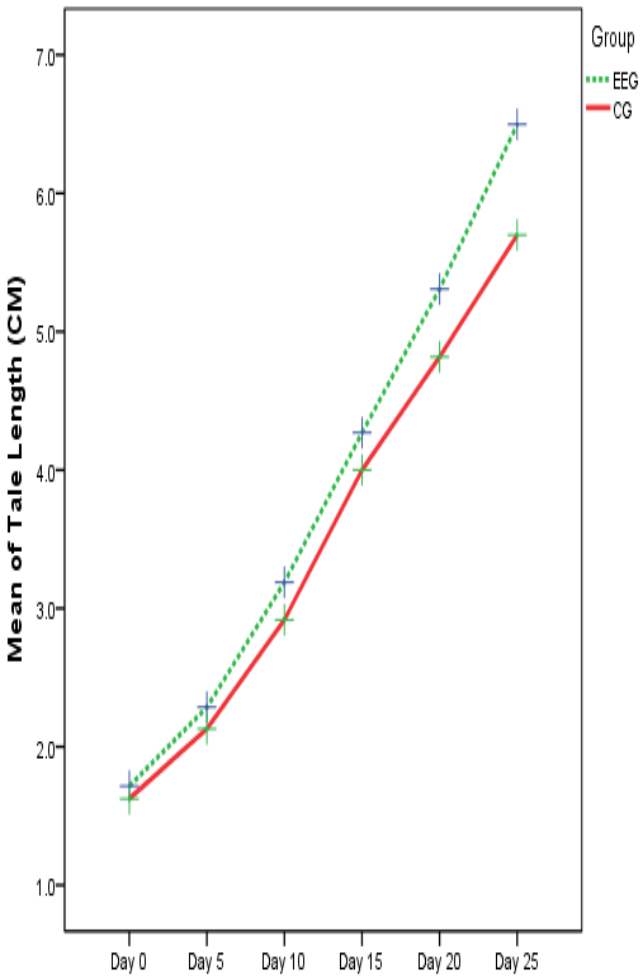

Figure 1. Mean plots of body weight, body length, and tail length. According to the ANOVA for Repeated Measurement, the body weight, body length, and tail length EEG offspring was statistically superior to the CG offspring (Table 5). Specifically, in the six observation times all the body weight of EEG were statistically heavier than the CG ($p<0.05$ Table 3) except day 20 ($p<0.05$ Table 5), all the body length of EEG were statistically longer than the CG ($p<0.05$ Table 5) except day 0 and day 20 ($p<0.05$ Table 5), and all the tail length of EEG were statistically longer than the CG ($p<0.05$ Table 5).

Table 5. Comparisons of the body weight, body length, and tail length between EEG and CG in different observing times.

Time (d)	EEG				CG			
	n	Body weight (g)	Body length (cm)	Tail length (cm)	n	Body weight (g)	Body length (cm)	Tail length (cm)
Day 0	141	6.22±0.62	5.00±0.24	1.72±0.18	65	5.47±0.67*	4.90±0.24*	1.63±0.15*
Day 5	117	8.66±1.44	5.83±0.51	2.29±0.32	37	8.09±1.10*	5.82±0.41	2.13±0.23*
Day 10	116	16.24±3.50	7.29±0.57	3.19±0.42	36	14.96±2.11*	6.90±0.48*	2.92±0.27*
Day 15	113	24.67±4.00	8.22±0.58	4.27±0.33	36	21.22±2.02*	7.81±0.34*	4.00±0.27*
Day 20	113	32.71±5.92	9.50±0.70	5.31±0.58	36	31.72±3.84	9.44±0.67	4.82±0.47*
Day 25	112	49.00±8.13	11.39±0.65	6.49±0.54	35	42.37±4.29*	10.54±0.47*	5.70±0.44*

* refers to $p<0.05$

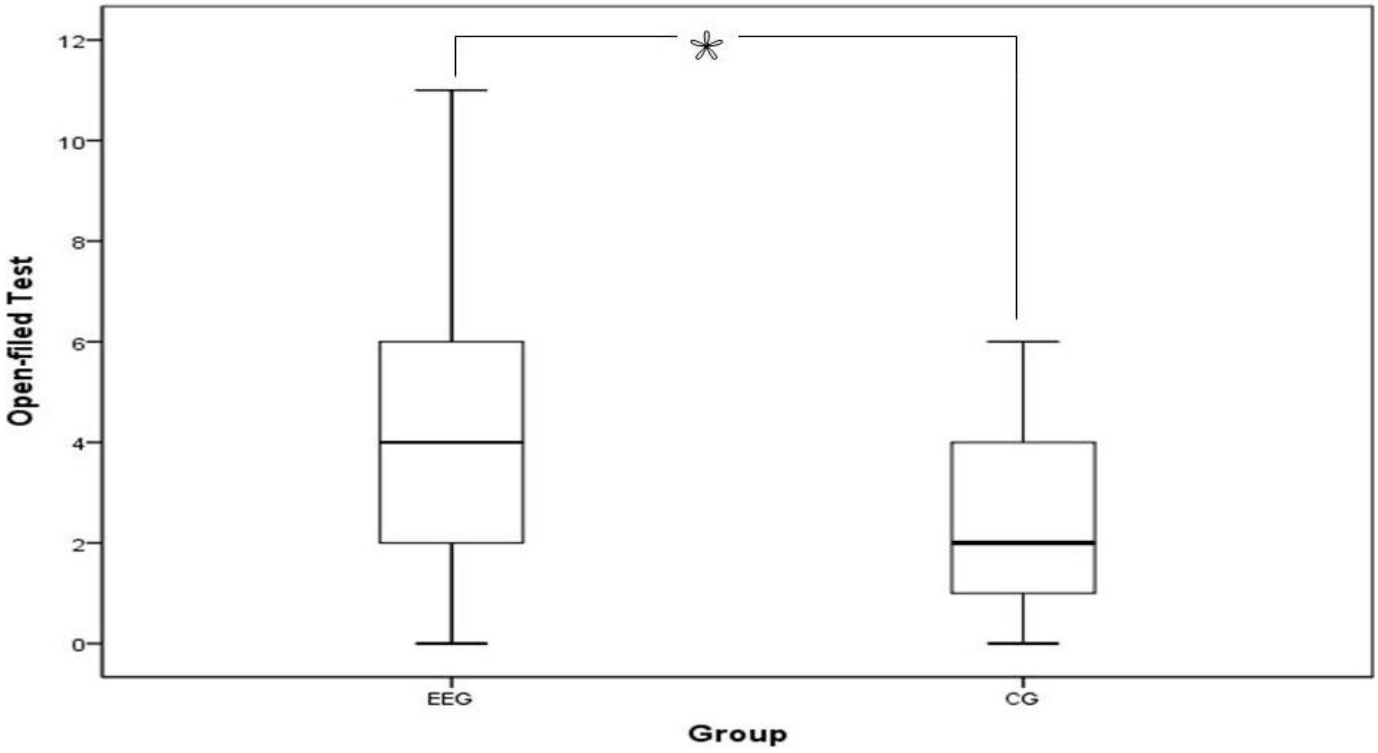

Figure 2. Box plot of Open-filed Test in the comparison between EEG and CG. EEG showed more cells than CG ($p<0.05$,*).

Table 6. ELISA outcomes of Cortisol, Dopamine, 5-HT, and Growth Hormone

Hormone	Group	n	Mean	Standard deviation	t	df	p
Cortisol	EEG	10	205.1130	30.00650	-3.40	18	0.738
	CG	10	213.0610	67.48351			
Dopamine	EEG	10	42.6830	2.07167	1.723	18	0.102
	CG	10	40.7151	2.95729			
5-HT	EEG	10	89.8601	17.16498	-0.684	18	0.503
	CG	10	94.0866	9.35450			
Growth Hormone	EEG	10	10.3127	1.33564	1.806	18	0.088
	CG	10	9.3251	1.09848			

and 23 down-regulated genes, from which five significant GO annotations were obtained including nitrogen compound metabolic process, thioredoxin-disulfide reductase activity, catabolic process, phosphate binding and hydrolase activity. These pathways have been shown to influence memory and learning (van Praag et al., 2000; Cheng et al., 2003; Hunter, 2000), and so these findings are consistent with previous research suggesting that EE can facilitate memory and learning by improving brain plasticity (Hunter, 2000).

Nitricoxide (NO) may act as an intercellular signal which regulates synaptic plasticity in mature neurons and regulates the proliferation and differentiation of mouse brain neural progenitor cells (Cheng et al., 2003). Phosphorylation has been proven to be a fundamental covalent posttranslational modification which regulates the function, localization and binding specificity of target proteins (Hunter, 2000). Protein phosphorylation plays an important role in triggering synaptic changes underlying learning and memory and recent studies highlight the importance of tightly regulated dephosphorylation as a mechanism controlling the induction of long-term synaptic change and lasting memory (Sweatt, 2001). Rats raised in EE condition have been found to perform better on a spatial memory task than rats raised in isolated condi-tions, which may result from alterations in NGF, BDNF and NT-3 protein levels in several brain regions (Ickes et al., 2000). Bruel-Jungerman et al. (2005) reported that EE could lead to improved long-term recognition memory and increased hippocampal neurogenesis. In addition,

Table 7. Differentially expressed genes in EEG vs CG, among which 25 genes were up-regulated and 23 genes down-regulated.

Gene ID	p-values	Fold change	Gene symbol	Expression
24981	0.006136	0.267452	RT1-Db2	Down-regulation
117095	0.006497	0.310416	Bhlhe41	Down-regulation
114248	0.007221	0.332688	Pde6h	Down-regulation
365948	0.003811	0.376363	Bank1	Down-regulation
293854	0.008224	0.385496	Srpk3	Down-regulation
24446	0.006543	0.424728	Hgf	Down-regulation
246307	0.002212	0.426579	Asrgl1	Down-regulation
499356	0.004756	0.427616	Blnk	Down-regulation
192270	0.009563	0.434192	Ppap2b	Down-regulation
116682	0.007003	0.445661	Kynu	Down-regulation
60586	0.002756	0.449476	Clcn4-2	Down-regulation
305070	0.00744	0.468121	Tmem206	Down-regulation
171047	0.004199	0.471599	RGD620382	Down-regulation
25314	0.006808	0.4801	Emp1	Down-regulation
362564	0.00575	0.528526	Bend5	Down-regulation
246324	0.006779	0.535022	Rab31	Down-regulation
29140	0.008248	0.569647	Snn	Down-regulation
299783	0.004571	0.583811	Glipr1	Down-regulation
293118	0.000742	0.592168	Prcp	Down-regulation
245966	0.004146	0.594673	Tmem150a	Down-regulation
301111	6.18E-05	0.656727	Ano10	Down-regulation
309373	0.007705	0.663006	Ubtd1	Down-regulation
501550	0.008809	0.795357	Trappc2	Down-regulation
680856	0.009565	1.165038	LOC680856	Up-regulation
360855	0.004549	1.17008	Smg7	Up-regulation
360853	0.007741	1.219983	Uchl5	Up-regulation
313418	0.009701	1.230535	Mier1	Up-regulation
366952	0.002191	1.246157	Cpsf1	Up-regulation
361732	0.008115	1.376306	Tmem109	Up-regulation
360784	0.009597	1.379661	Znhit1	Up-regulation
308267	0.008796	1.382562	Map3k7ip2	Up-regulation
78957	0.008023	1.423074	Shank1	Up-regulation
50522	0.007162	1.423922	Ubc	Up-regulation
503165	0.007864	1.434636	LOC503165	Up-regulation
310859	0.009689	1.440774	Tet2	Up-regulation
81650	0.001523	1.507886	Csnk2b	Up-regulation
116484	0.005497	1.557774	Txn1	Up-regulation
497991	0.002311	1.565045	Med1	Up-regulation
360903	0.008192	1.627706	Fgfrl1	Up-regulation
59319	0.00161	1.694855	Nyw1	Up-regulation
298884	0.007543	1.702151	Gen1	Up-regulation
300208	0.002839	1.816253	Ddx23	Up-regulation
79430	0.00281	2.197877	Clcnkb	Up-regulation
308543	0.006159	2.373522	Plekhf1	Up-regulation
24916	4.57E-06	2.445061	Rnf112	Up-regulation
24769	0.000216	2.78425	Sct	Up-regulation
294284	0.003997	2.972357	AA926063	Up-regulation
311598	0.004196	3.047816	Fam83d	Up-regulation

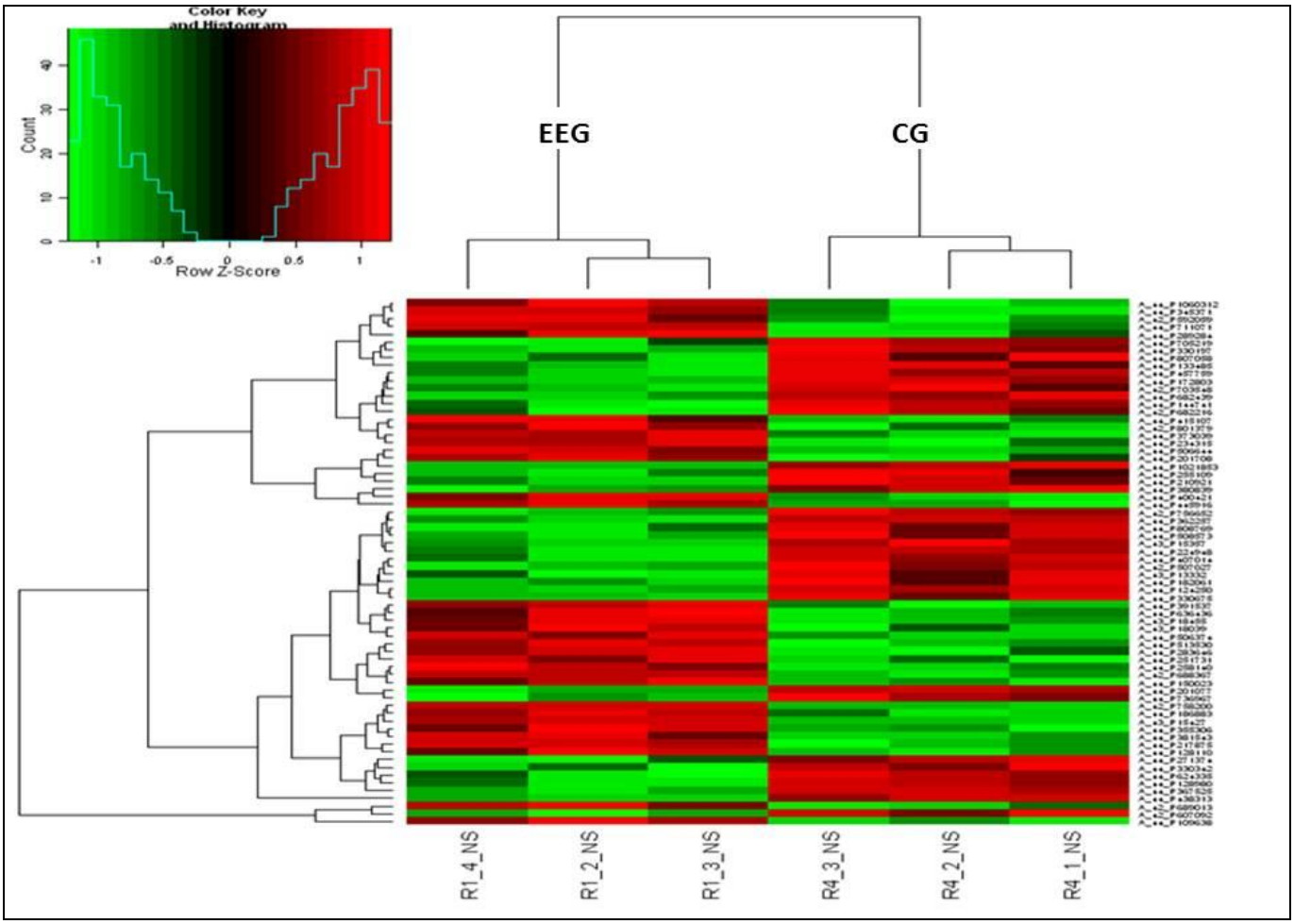

Figure 3. Heat-map of the differentially expressed genes. R1_2_NS, R1_3_NS, R1_4_NS refer to RRG and R4_1_NS, ~R1_4_NS refer to CG. The heat-map showed EEG and CG were clearly seperated.

Table 8. Significant GO Annotation of the 48 differentially expressed genes and the genes included (p<0.05).

GO ID	Name	Symbol	Hits	Total	%	Enrichment Test p-value
GO:0006807	nitrogen compound metabolic process	Kynu, Asrg l1,G6pc Med1	5	397	1.26	0.0071
GO:0004791	thioredoxin-disulfide reductase activity	Txn1	1	6	16.67	0.0209
GO:0009056	catabolic process	Kynu,RGD620382 Tmem150,Asrg l1 G6pc,Uchl5,Ubc	7	933	0.75	0.022
GO:0042301	phosphate binding	G6pc	1	9	11.11	0.0298
GO:0016787	hydrolase activity	Pde6h,Kynu RGD620382,Ppap2b Hgf,Asrg l1 Rnf 112,G6pc,Prcp Ddx23,Uchl5	11	1967	0.56	0.0315

Table 9. KEGG Pathway annotation of the 48 differentially expressed genes (p<0.05, q<0.05) (↓refers down-regulation, ↑refers up-regulation).

Name	Symbol	Total	%	p	q
Circadian rhythm	Dec ↓	13	7.69	0.0195	0.0246
Ether lipid metabolism	Ppat2b2↓	33	3.03	0.0468	0.0246
Endocytosis	Rab22↓ Arfgap35↑	240	0.83	0.0458	0.0246
Primary immunodeficiency	BLNK↓	35	2.86	0.0495	0.0246

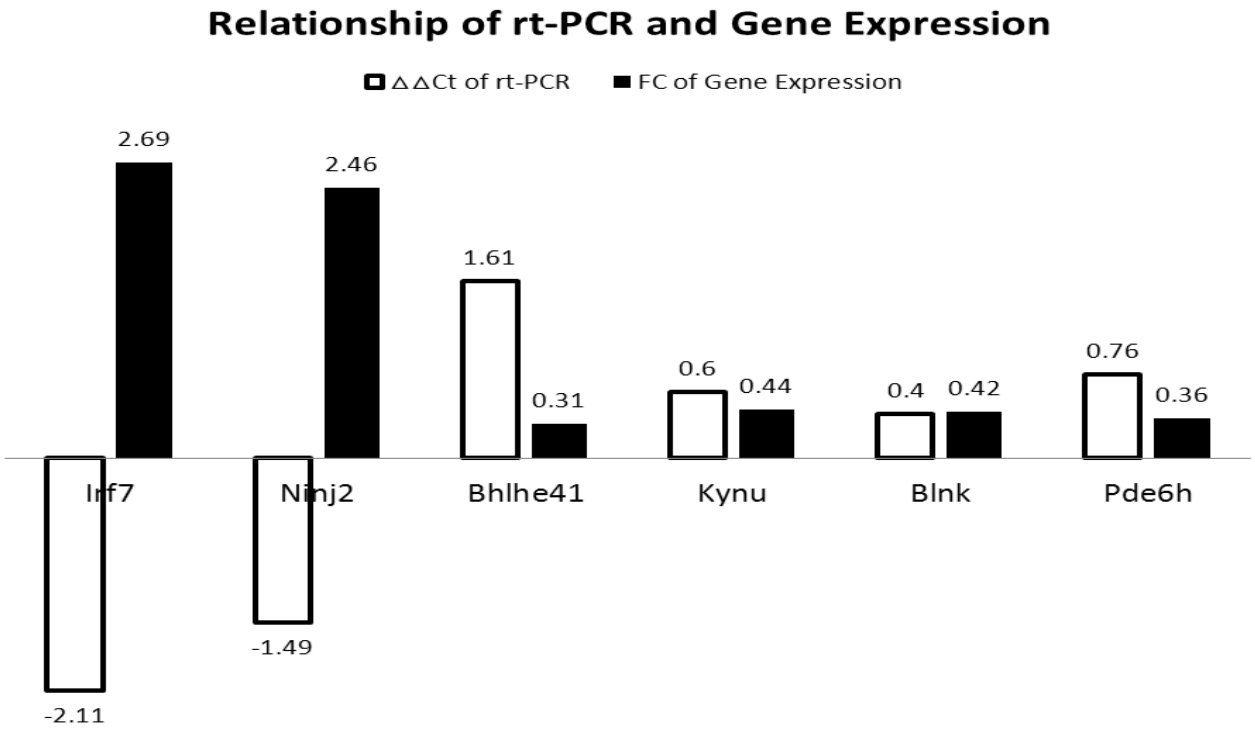

Figure 4. RT-PCR validation of the selected six genes from gene expression profile chips, i.e. Irf7, Ninj2, Bhlhe41, Kynu, Blnk, and Pde6h. △△Ct<0 indicates the target genes were hyper-expressed in EEG comparing with CG while △△Ct>0 indicates the targeted genes were hypo-expressed in EEG comparing with CG. FC>2 indicates the target genes were hyper-expressed in EEG comparing with CG while FC<2 indicates the target genes were hypo-expressed in EEG comparing with CG.

abnormal protein phosphorylation is deeply related to neuropathogenesis.

Changes in four major KEGG pathways were annotated within EE group, including pathways related to circadian rhythm maintenance, ether lipid metabolism, endocytosis and primary immunodeficiency. Circadian rhythms are common to most organisms and govern much of the homeostasis and physiology (Hatanaka et al., 2010; Holme et al., 2004). Although, maternally influenced circadian rhythm antenatally becomes ultradian at birth (Mirmiran, et al., 2003). Ether lipid is ubiquitous and sometimes major parts of the cell membranes in mammals, which acts as second messenger and also act directly in cell signaling involved in leukocyte function in the mammalian immune system (Paltauf, 1994; Demopoulos et al., 1979). EE has been previously described as enhancing natural killer cell activity of recognizing and killing virally infected cells (Benaroya-Milshtein et al., 2004). Rats rose under EE present immune cell recruitment with a higher number of activated microglia than control rats, and these ramified microglial cells resemble the neuro-protective phenotype of microglia activated by T-cell–derived cytokines (Ziv et al., 2006).

de Sousa et al. (2011) reported that EE promotes a more effective immune response to clear central nervous system (CNS) virus and not at the cost of CNS damage. Williamson et al. (2012) reported that EE rats had a markedly blunted pro-inflammatory response to lipopolysaccharide within the hippocampus, and the pro-inflammatory cytokine IL-1β were all significantly decreased following lipopolysaccharide administration in EE rats compared to controls. Six genes that is, Irf7, Ninj2, Bhlhe41, Kynu, Blnk and Pde6h were validated with RT-PCR. Vary components of the kynurenine (Kynu) pathway

of tryptophan metabolism are now recognized to harbor profound biological importance including the ability of modulating the activation of glutamate and nicotinic receptors, modifying the responsiveness of the immune system to inflammation and infection, and modifying the generation and removal of reactive oxygen species (Stone et al., 2012). Each of the aforementioned factors has been recognized as contributor to major disorders of the central nervous system, therefore the potentially fundamental role of the kynurenine pathway in those disorders demonstrates a promising valuable target both for understanding the mechanism of those disorders and for developing potential drug treatments (Vamos et al., 2009). Bhlhe41 also called Dec2, Sharp1, Bhlhb3 and Bhlhb2l may play a role in regulating neuronal differentiation during development and adaptive neuronal plasticity and neurite outgrowth (Yang et al., 2009).

Of the basic helix-loop-helix (bHLH) proteins which bind to the E box sequence, class B of the proteins, BHLHB2 and BHLHB3 are transcription factors which contain a unique orange domain (Fausett et al., 2008). Their gene expressions are regulated in a cell type-specific manner by various extracellular stimuli such as growth factors, serum starvation, hypoxia, hormones, nutrient, cytokines, light and infection. Therefore, these transcription factors play pivotal roles in multiple signaling pathways that impact many biological processes including development, cell differentiation, cell growth, cell death, oncogenesis, immune systems, circadian rhythm and homeostasis (Yamada and Miyamoto, 2005). The adaptor protein Blnk binds various signaling proteins and immune globin, a signaling subunit of the cross-linked B cell receptor complex, and plays an essential role in the B cell receptor signal transduction, which was shown to be required to activate ERK, but not of Ras, after B cell receptor cross-linking (Imamura et al., 2009).

It is reported that all elements of IFN responses, whether the systemic production of IFN in innate immunity or the local action of IFN from plasmacytoid dendritic cells in adaptive immunity are under the control of Irf7 (Honda et al., 2005). Hannah et al. (2008) reported that induction of pattern recognition receptors (PRRs; Tlr7 and Rig-I), expression of antiviral genes (Myd88, Visa, Jun, Irf7, Ifnβ, Ifnar1, Jak2, Stat3, and Mx2), and production of Mx protein were elevated in the lungs of intact females compared with intact males (Araki et al., 2000). Ninjurin2 (Ninj2) is a transmembrane protein that mediates cell-to-cell and cell-to-extracellular matrix interactions during development, differentiation and regeneration of the nervous system (Lin et al., 2011). Recently, Nunj2 was reported to be a vascular susceptibility gene and associated with Alzheimer's disease risk (Wang et al., 2004). Pde6h may play a role in mitogen activated protein kinase signaling (Morin et al., 2003).

Conclusion

Together with our own recent data, the findings of this work demonstrate that the environmental enrichment (EE) of prenatal rats during pregnancy could positively promote the body and nervous system development. Although, this study did not find evidence that neurohormones mediate these effects, environmental enrichment was associated with an altered gene expression profile. The gene pathways involved have been tied to circadian rhythm and immune process, and previous research has related disruptions in such systems to EE. This study demonstrates that positive prenatal experiences have the ability to significantly alter offspring developmental and immunity trajectories.

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