Brief communication

Early repeated maternal separation induces alterations of hippocampus reelin expression in rats

JIANLONG ZHANG†, LINA QIN† and HU ZHAO*

Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510080, China

**Corresponding author (Fax, +86-20-87334353; Email, hzhao@163.com)*

† These authors contributed equally to this work.

The long-term effects of repeated maternal separation (MS) during early postnatal life on reelin expression in the hippocampus of developing rats were investigated in the present study. MS was carried out by separating Wistar rat pups singly from their mothers for 3 h a day during postnatal days (PND) 2–14. Reelin mRNA and protein levels in the hippocampus were determined using qRT-PCR and Western blotting, at PND 22, PND 60 and PND 90. MS resulted in the loss of body weight in the developing rats, and reelin mRNA and protein levels in the hippocampus generally were down-regulated over the developing period, but the reelin mRNA and protein levels in the hippocampus of 90-day-old male rats were up-regulated. These findings suggest that the long-term effects of MS on the expression levels of hippocampal reelin mRNA and protein depends on the age at which the stressed rats' brains were collected; reelin had important implications for the maternal-neonate interaction needed for normal brain development. In conclusion, repeated MS occurring during early postnatal life may cause the alterations of hippocampal reelin expression with the increasing age of developing rats.

[Zhang J, Qin L and Zhao H 2013 Early repeated maternal separation induces alterations of hippocampus reelin expression in rats. *J. Biosci.* **38** 27–33]
DOI 10.1007/s12038-012-9286-3

1. Introduction

Maternal separation (MS) is accepted to be a stressful condition in rodents, presumably resulting in disturbance in normal brain development. The exposure to stress during neurodevelopment has an impact on both physical and mental health (Fumagalli *et al.* 2007; Gluckman *et al.* 2008). Both human and animal studies suggest that early-life stress has profound effects on neuron function and emotional health. The hippocampus is known as one of the brain regions which is vulnerable to stress (Vermetten and Bremner 2002). Interestingly, it mediates, and in turn is affected by stress responses. Moreover, the hippocampus plays an important role in learning and memory. Clinical and laboratory investigations indicate that MS, during the critical periods of brain development, can lead to disruption of hippocampal cytoarchitecture, contributing to learning disability and behavioral abnormalities (Matthews *et al.* 1996; Huot *et al.* 2002).

In the adult hippocampus, reelin expression occurs in interneurons residing primarily in the hilar region of dentate gyrus, and the stratum lacunosum-moleculare layer of the hippocampus proper (Pesold *et al.* 1998). The characteristic function of reelin is the control of radial neuronal migration and the formation of cellular layers during prenatal brain development. In recent years, it has become clear that reelin not only controls neuronal migration during embryogenesis, but also promotes neuronal maturation and functions at postnatal ages. Reelin functions in the postnatal developing and adult hippocampus by affecting synaptic strength and plasticity (Qiu *et al.* 2006; Qiu and Weeber 2007). The postnatal functions of reelin appear to be mediated by the same signalling pathway that mediates its function in neuronal migration (Niu *et al.* 2004). These studies demonstrate

Keywords. Hippocampus; maternal separation; real-time PCR; reelin mRNA

Published online: 16 December 2012

the importance of reelin in the formation and maintenance of the central nervous system.

In the rat, increased maternal licking/grooming and arched-back nursing behaviour over the first week of life is associated with enhanced hippocampal neuronal survival, synaptogenesis and improved cognitive performance under stressful conditions (Liu *et al.* 2000; Weaver *et al.* 2002). These findings suggest a rather extensive influence of maternal care on hippocampal gene expression. Weavers' study (Weaver *et al.* 2006) indicated that the hippocampal reelin expression was significantly greater in the methionine-treated offspring of mothers with increased licking/grooming and arched-back nursing in comparison to the vehicle-treated offspring of mothers with decreased licking/grooming and arched-back nursing. This evidence indicates that reelin gene expression might be changed in the hippocampus after MS. However, investigations into the transcription-regulating mechanisms mediating changes of reelin gene expression in MS rat pups are lacking, partly because of the complex structure of the reelin gene. Therefore, we decided to examine the effects of repeated MS on the reelin expression in hippocampus during early postnatal life by using real-time PCR.

2. Materials and methods

2.1 *Animals and maternal deprivation*

Primiparous pregnant female Wistar rats were used in the present study. They were kept in a room with a constant temperature (23±2°C) and maintained in a 12/12 h light/dark cycle (lights on at 8:00). Food and water were available *ad libitum*. All experimental protocols described in this study were approved by the Local Institutional Animal Care and Use Committee.

MS was performed according to a previously described method (Rosztoczy *et al.* 2003; Barreau *et al.* 2004). The birth day was designated as PND 1. After delivery (day 1), litters were then randomly assigned to either MS group or mother-reared control (MRC) group. MS manipulation was performed once daily for three consecutive hours (from 9:00 to 12:00). Each pup in MS group was removed from their home cage and kept singly in temperature controlled cages at 28±1°C. This procedure was applied during PND 2-14. The rat pups of the MRC group were left undisturbed with their dam. All the rat pups were weaned on day PND 22, and housed 6 animals per cage in same sex/same litter group until PND 90.

2.2 *Sample collection*

Animals were brought into a separate room and sacrificed on PND 22, PND 60, and PND 90 by decapitation. The brain was rapidly removed, and the hippocampus immediately dissected and frozen at −70°C for further experiments.

2.3 *Real-time reverse transcription quantitative PCR*

RNA was isolated from hippocampus tissue and purified by RNAqueous Kit (Part Number AM1912, Ambion). qRT-PCR was performed using commercially available reagents (High-Capacity cDNA Reverse Transcription Kits, Applied Biosystems). For a 20 μL reaction mixture, the following reagents were included: 2 μL of 10×RT buffer, 0.8 μL of 100 mM 25×dNTP mix, 1 μL of MultiScribe™ reverse transcriptase, 1 μL of RNase inhibitor (40 unit/ μL), 2 μL 10×RT random primers, 3.2 μL of RNase-free water and 10 μL of sample RNA. Reverse transcription was conducted at 25°C for 10 min, followed by 37°C for 120 min, and 85°C for 5 min, and then stored at −80°C. Reelin mRNA levels were measured by qRT-PCR using commercially available reagents Power SYBR® Green PCR Master Mix (Applied Biosystems). All primes were designed to span exon boundaries, ensuring amplification of only mRNA. The following forward and reverse primers were used in the present study: 5′GAGTCCACTATACAACCAGA3′, forward primer and 5′TGATCGAAAGCAGAGACGTC3′, reverse primer. Product purity was confirmed by melting curve analysis and agarose gelel ectrophoresis in the presence of ethidium bromide. Relative gene expression comparisons were carried out using 18S RNA as an invariant endogenous control. Initial mRNA copy numbers in each sample were calculated according to the $\Delta\Delta C_T$ method (Livak and Schmittgen 2001; Pfaffl 2001). Data was expressed as C_T values and used to determine ΔCT values (ΔC_T=C_T of the target gene – C_T of the housekeeping gene), which was calculated for each sample. The control sample was used as the baseline for each comparison to be made. The last step in quantification was transformation of these values into absolute values. The formula for fold changes in gene expression is $2^{-\Delta\Delta CT}$. Statistics were performed with ΔCT values. Reactions were performed in a 20 μL volume with 5 μL of the cDNA, 10 μL of SYBR Green Supermix (Applied Biosystems), 1 μL of primer, and 4 μL of Depc H_2O. The amplification protocol consisted of one cycle at 95°C for 10 minutes, followed by 35 cycles at 95°C for 15 s, 60°C for 15 s, 72°C for 20 s, and 90°C for 2 s. Detection of the fluorescent products were carried out at the end of the 90°C extension period. All qRT-PCR reactions were performed in triplicate.

2.4 *Western blot*

Total protein was extracted from the hippocampal tissues using RIPA lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA). First, 40 μg of lysate was subjected to 12%

SDS PAGE (Bio-Rad, Hercules, CA, USA), transferred to polyvinylidene difluoride membranes (Millipore), and incubated for 2 h with a 1:700 dilution of anti-reelin monoclonal antibody (MAB5364, Millipore, USA), or anti-β-actin antibody (Santa Cruz Biotechnology). Secondly, the membranes were washed and treated with goat anti-mouse Ig G (1:2,000, Santa Cruz Biotechnology) for 1 h at room temperature. Lastly, optical densities (ODs) of the individual bands were visualized using Image Pro Plus 6.0 for Windows. All protein blots were normalized to actin blots for each individual lane; duplicates of each sample were examined in separated membranes. The final results are averages of two independent duplicates.

2.5 *Statistical analysis*

The data was expressed as mean±S.E.M. *t*-Tests were used for the comparison of the body weight. Two-way ANOVA analysis of variance was used to analyse all other data. Significance was set at $P<0.05$ for all tests. The statistical analysis was done using the SPSS Statistical Software version 16.0.

3. Results

3.1 *Somatic development*

Body weights in the control and MS rats at PND 22, PND 60, and PND 90 are shown in figure 1. The results showed that the average body weight of MS rats were lower than that of control animals of both male and female during the whole period ($n=8$). There were significant differences between control and MS rats at PND 22 ($t=8.988$, df=14, $P<0.001$; $t=5.917$, df=14, $P<0.001$, male and female respectively), PND 90 ($t=5.055$, df=14, $P<0.001$; $t=4.306$, df=14, $P<0.001$, male and female respectively), and PND 60 only in females ($t=6.205$, df=14, $P<0.001$ for female; $t=1.388$, df=14, $P>0.05$ for male).

3.2 *Variation of reelin mRNA expression in hippocampus*

Real-time quantitative PCR was used to evaluate changes of reelin mRNA expression levels in the developing hippocampus. The reelin gene expression values were expressed as the comparative threshold cycle ($2^{-\Delta\Delta CT}$) method. Thus, the smaller the C_T value, the higher the gene expression levels, namely, the gene expression levels were consistent with $2^{-\Delta\Delta CT}$. The greater the $2^{-\Delta\Delta CT}$, the higher the gene expression levels. Our results about $2^{-\Delta\Delta CT}$ of reelin were shown in figure 2 ($n=6$). There were downtrends of reelin mRNA expression in MS group compared to control rats except for the 90-day-old male rats. Two-way ANOVA revealed a significant group by age interaction ($F_{(2,20)}=4.645$, $P=0.022$; $F_{(2,20)}=1.319$, $P=0.290$, male and female respectively), age×group ($F_{(2,20)}=8.305$, $P=0.002$; $F_{(2,20)}=0.141$, $P=0.869$, male and female respectively), and significant effect of intercept group ($F_{(1,10)}=9.064$, $P=0.013$; $F_{(1,10)}=10.729$, $P=0.008$, male and female respectively). Significant main effect of age was found only in male rats but not in female rats. Post-hoc analysis showed that reelin mRNA level was significantly lower in 60-day-old rats than that of 22-day-old male rats ($P<0.05$). Collectively, repeated MS had significant effects on hippocampal reelin mRNA expression. The main effect of age was observed only in male rats between PND 22 and PND 60.

3.3 *Variation of reelin protein expression in hippocampus*

As seen in figure 3, reelin protein can be confirmed by western blotting analysis. Two-way ANOVA revealed significant group by age interaction in reelin protein expression ($F_{(2,20)}=7.016$, $P=0.005$; $F_{(2,20)}=5.207$, $P=0.015$, male and female respectively). There was a significant main effect between MS and control rats ($F_{(1,20)}=4.552$; $P<0.05$, $F_{(1,20)}=7.545$, $P<0.05$, male and female respectively). Lower expression levels of reelin protein in MS rats were observed compared to control groups except for the 90-day-old male rats which had similar values to controls.

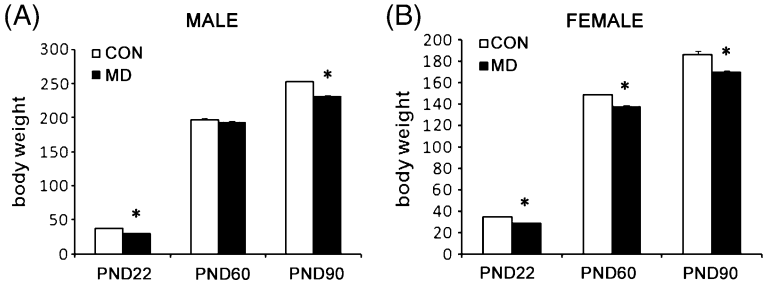

Figure 1. The changes of body weight in MS rats and control. (**A**) Average body weights of MS rats decreased significantly versus control rats at PND 22 and PND 90 of males (*$P<0.05$). (**B**) There were significant differences of average body weights between control and MS rats at PND 22, PND 60 and PND 90 of females (*$P<0.05$). All results are expressed as mean±S.E.M. ($n=8$ rats per group).

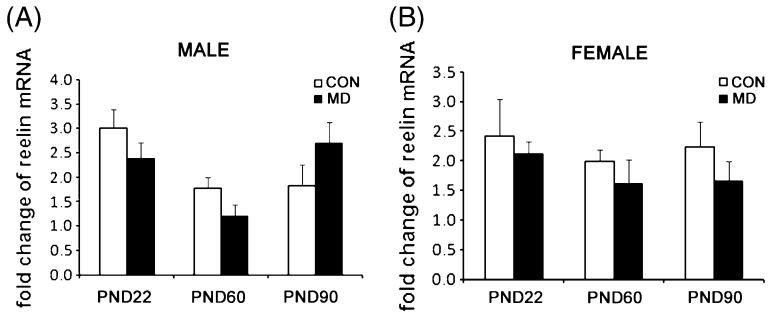

Figure 2. The expression of reelin mRNA in the hippocampus of male and female rats.The value for reelin mRNA was normalized by that for the internal standard 18S mRNA. Panel **A** shows male and Panel **B** shows female groups. Two-way ANOVA revealed a significant group by age interaction ($P<0.05$, both male and female rats), and significant main effect of group ($P<0.05$, both male and female rats). There was only significant main effect of age in male rats but not in female rats. Each column represents the mean±S.E.M. ($n=6$ rats per group).

4. Discussion

The reelin expression by specialized Cajal-Retzius cells, located in the marginal zone, is required for the ventricular migrating neurons to form a highly laminated structure in the neocortex, hippocampus and cerebellum, which is indispensable for the formation of a functional network and activity-driven synaptogenesis (D′Arcangelo *et al.* 1995; Soda *et al.* 2003). However, persistent expression of reelin in the hippocampus after the completion of cell migration is still required for proper function of synaptic transmission and plasticity. This signalling system profoundly modifies mammalian learning and memory behavior (Beffert *et al.* 2005; D′Arcangelo 2005). Numerous studies have shown that reeler mice develop profound cognitive deficits that are similar to those observed in human lissencephaly patients bearing a *RELN* gene mutation (Hong *et al.* 2000).

We previously demonstrated that long-lasting predatory stress may induce the neuronal cell loss in hippocampus (Zhao *et al.* 2007). It was believed that the neuronal loss was one of the important factors involved in the reduction of hippocampal volume (Sapolsky 2000). It was known that glucocorticoids could affect structural and functional development in the hippocampus via influence of the balance between neurogenesis and neuronal cell death (Gould *et al.* 1991). A recent study revealed a significant decrease in the number of reelin-positive cells in the CA1 stratum lacunosum and the subgranular zone of the dentate gyrus in rats that received corticosterone (Lussier *et al.* 2009). The changes of reelin expression level in the hippocampus during critical developmental windows would affect hippocampus functions, ultimately leading to learning disabilities, behavioural abnormalities or even psychiatric disorders (D′Arcangelo *et al.* 1995; Weaver *et al.* 2002). The heterozygous reeler mouse (HZ) revealed important differences which were consistent

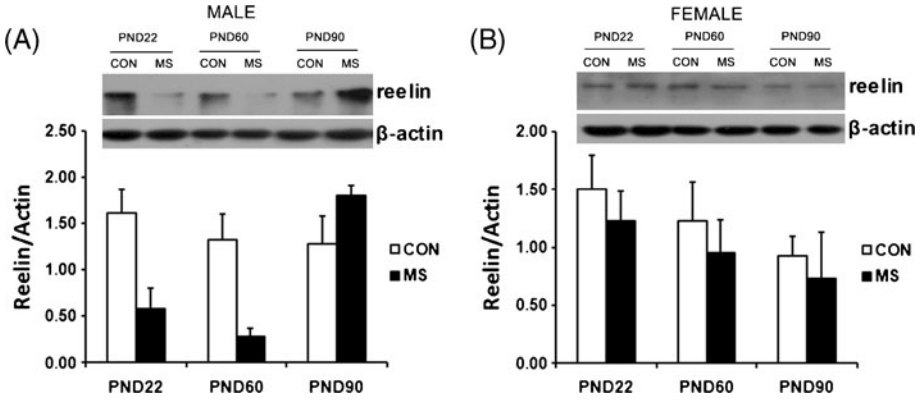

Figure 3. The expression reelin protein in the hippocampus of male and female rats. Comparison of reelin protein expression levels in the hippocampus between control and MS rats at PND 22, PND 60 and PND 90. Two-way ANOVA revealed significant group by age interaction in expression amount of reelin protein ($P<0.05$). There were significant main effects of group and age between MS and control rats ($P<0.05$, both in male and female rats). Panel **A** shows male representative immunoblots and Panel **B** shows female representative immunoblots. Each column represents the mean±S.E.M. ($n=4$ rats per group).

with decreased behavioral inhibition and emotionality. An HZ mouse representative would make an interesting animal model for behavioral abnormalities seen in diseases like autism and schizophrenia (Laviola *et al.* 2009). The present results strongly indicate that 3 h daily MS for 2 weeks may induce significant down-regulation of hippocampus reelin levels compared with control groups in the developing hippocampus. Therefore, it might be speculated that repeated MS exerted harmful effects on hippocampus development and function through glucocorticoid-mediated decrease of reelin expression.

In the previous reports, several-hour-long maternal separations resulted in significantly less weight gain (Huot *et al.* 2002; Barna *et al.* 2003). Our results showed that the maternal separation groups displayed reduction of body weight at PND 22, PND 60 and PND 90, and the weight loss induced by maternal separation continued into adulthood. Maternal separation resulted in a moderate decrease in body weight in wild-type and reeler pups (Ognibene *et al.* 2007). These earlier results were consistent with our present data.

Many experimental results in gender differences could help to better understand the sex differences in the occurrence, prevalence and severity of stress-induced disorders in various fields of neuroscience. In the present study, males showed a trend of up-regulation in mRNA and protein at PND 90 of MS group, while females displayed a trend of down-regulation during the same period. The data suggest that by PND 90 the male rats compensate at least in terms of reelin expression. The males showed no difference of weight at PND 60 between MS and control groups, which indicated that the nervous system in males was not as sensitive as that in females in some respects. However, male and female reactions to early MS were only subtly different. We believe that those differences might be considered as gender-dependent changes due to the long-lasting sex-different effects of MS. An interaction between altered reelin function and altered estrogen/androgen balance could be an important pathophysiological mechanism in autism spectrum disorder (ASD), as well as other psychoses. These connections could be a critical target where reelin down-regulation and hormone levels could converge to generate social and cognitive deficits (Macri *et al.* 2010). Furthermore, an extensive amount of literature has shown differential regulation by gonadal hormones, resulting in established sex differences in proliferation and survival (Falconer and Galea 2003; Galea *et al.* 2006). Meanwhile, rats were sensitive to early life stress, especially before their adulthood. The relationship between quality of early life and health at adulthood is still an open question. Stressful events experienced early during postnatal life can influence the development of individual differences in vulnerability to psychopathology throughout life (Heim and Nemeroff 2001). During the early postnatal phases the brain is experience-seeking and provided with a considerable plasticity which allows a fine tuning between the external environment and the developing organism (Greenough 1987). One possible hypothesis posed is that adversity early in life is able to enhance or inhibit the experience-dependent maturation of structures underlying emotional functioning and endocrine responses to stress, such as the cortico-limbic system, leading to increased stress responding at adulthood (Tronick and Weinberg 1997; Heim *et al.* 2000; Schore 2000; Heim and Nemeroff 2001; Meaney 2001; Seckl and Meaney 2004; Cirulli *et al.* 2009). Both the present and previous findings can mainly be ascribed to a possible compensatory mechanism whereby when maternal separation was applied postnatal, these compensatory systems were primed so that stress later in life led to responses reflecting an overly protective mechanism. This was plausible as corticosterone administration in early postnatal life, as would be the case with maternal separation, has been shown to cause alteration of neurotrophins and their receptors such as reelin expression in the hippocampus (Roskoden *et al.* 2004; Faure *et al.* 2007). Our previous study showed that on PND 22, the expression of reelin mRNA reduced in the hippocampus followed by MD. Meanwhile, the changes of DNA methylation showed an opposite trend compared with the reelin expression. The results suggest that MD in early life has harmful effects on neurobehavioural development, and causes the down-regulation of reelin mRNA by further DNA methylation in postnatal hippocampus (Qin *et al.* 2011). In view of the fact that early-life-experience-related disorders are associated with profound behavioral alterations, it is tempting to speculate that both a reduced level of reelin and early stress exposure may contribute to dysfunction of hippocampus and possibly to increased vulnerability to stress-related disorders.

In conclusion, the present study showed that MS, as an early life stress, may result in the loss of body weight in rats, and cause the change of reelin mRNA expression in the three observation periods. There was a similar trend in reelin protein. On the whole, the expression levels of reelin mRNA and protein in the hippocampus would be down-regulated by repeated MS over the developing period. Our data also indicted that the effects of MS on the expression levels of reelin mRNA was age-dependent only in male rats. Meanwhile, MS rats had lower expression levels of reelin mRNA and protein compared with control groups except for the 90-day-old male rats. There were significant main effects of group by age between MS and control rats. These findings suggested that reelin had important implications for the maternal-neonate interaction for normal brain development. The reelin expression in different subregions of developing hippocampus need to be examined, and the critical influence of early environment on different developmental stages are required to be illustrated in future research.

Acknowledgements

This work was supported by 100 Talents Program of Sun Yat-sen University, China (5000-3171314) and National Natural Science Foundation of China (No. 81273350).

References

Barna I, Balint E, Baranyi J, Bakos N, Makara GB and Haller J 2003 Gender-specific effect of maternal deprivation on anxiety and corticotropin-releasing hormone mRNA expression in rats. *Brain Res. Bull.* **62** 85–91

Barreau F, Ferrier L, Fioramonti J and Bueno L 2004 Neonatal maternal deprivation triggers long term alterations in colonic epithelial barrier andmucosal immunity in rats. *Gut* **53** 501–506

Beffert U, Weeber EJ, Durudas A, Qiu S, Masiulis I, Sweatt JD, Li WP, Adelmann G, Frotscher M, Hammer RE and Herz, J 2005 Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. *Neuron* **47** 567–579

Cirulli F, Francia N, Berry A, Aloe L, Alleva E and Suomi SJ 2009 Early life stress as a risk factor formental health: role of neurotrophins from rodents to non-human primates. *Neurosci. Biobehav. Rev.* **33** 573–585

D′Arcangelo G 2005 Apoer2: a reelin receptor to remember. *Neuron* **47** 471–473

D′Arcangelo G, Miao GG, Chen SC, Soares HD, Morgan JI and Curran T 1995 A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature* **374** 719–723

Falconer EM and Galea LA 2003 Sex differences in cell proliferation, cell death and defensive behavior following acute predator odor stress in adult rats. *Brain Res.* **975** 22–36

Faure J, Uys JD, Marais L, Stein DJ and Daniels WM 2007 Early maternal separation alters the response to traumatization: resulting in increased levels of hippocampal neurotrophic factors. *Metab. Brain Dis.* **22** 183–195

Fumagalli F, Molteni R, et al. 2007 Stress during development: Impact on neuroplasticity and relevance to psychopathology. *Prog. Neurobiol.* **81** 197–217

Galea LA, Spritzer MD, Barker JM and Pawluski JL 2006 Gonadal hormone modulationof hippocampal neurogenesis in the adult. *Hippocampus* **16** 225–232

Gluckman PD, Hanson MA, Cooper C and Thornburg KL 2008 Effect of in utero and early-life conditions on adult health and disease. *New Engl. J. Med.* **359** 61–73

Gould E, Woolley CS, Cameron HA, Daniels DC and McEwen BS 1991 Adrenal steroids regulate postnatal development of the rat dentate gyrus: II Effects of glucocorticoids and mineralocorticoids on cell birth. *J. Comp. Neurol.* **313** 486–493

Greenough W 1987 Experience effects on the developing and the mature brain: dendritic branching and synaptogenesis; in *Perinatal development a psychobiological perspective* (eds) NA Krasnegor, EM Blass, MA Hofer and WP Smotherman (Orlando: Academic Press) pp 195–221

Heim C and Nemeroff CB 2001 The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol. Psychiatry* **49** 1023–1039

Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, Miller AH and Nemeroff CB 2000 Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* **284** 592–597

Hong SE, Shugart YY Huang DT, Shahwan SA, Grant PE, Hourihane JO, Martin ND and Walsh CA 2000 Autosomal recessive lissencephaly with cerebellar hypoplasia is associated with human RELN mutations. *Nat. Genet.* **26** 93–96

Huot RL, Plotsky PM, Lenox RH and McNamara RK 2002 Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res.* **950** 52–63

Laviola G, Ognibene E, Romano E, Adriani W and Keller F 2009 Gene-environment interaction during early development in the heterozygous reeler mouse: clues for modelling of major neurobehavioral syndromes. *Neurosci. Biobehav. Rev.* **33** 560–572

Liu D, Diorio J, Day JC, Francis DD and Meaney MJ 2000 Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat. Neurosci.* **3** 799–806

Livak KJ and Schmittgen TD 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25** 402–408

Lussier AL, Caruncho HJ and Kalynchuk LE 2009 Repeated exposure to corticosterone, but not restraint, decreases the number of reelin-positive cells in the adult rat hippocampus. *Neurosci. Lett.* **460** 170–174

Macri S, Biamonte F, Romano E, Marino R, Keller F and Laviola G 2010 Perseverative responding and neuroanatomical alterations in adult heterozygous reeler mice are mitigated by neonatal estrogen administration. *Psychoneuroendocrinology* **35** 1374–1387

Matthews K, Wilkinson LS and Robbins TW 1996 Repeated maternal separation of preweanling rats attenuates behavioral responses to primary and conditioned incentives in adulthood. *Physiol. Behav.* **59** 99–107

Meaney MJ 2001 Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu. Rev. Neurosci.* **24** 1161–1192

Niu S, Renfro A, Quattrocchi CC, Sheldon M and D′Arcangelo G 2004 Reelin promotes hippocampal dendrite development through the VLDLR/ApoER2-Dab1 pathway. *Neuron* **41** 71–84

Ognibene E, Adriani W, Macri S and Laviola G 2007 Neurobehavioural disorders in the infant reeler mouse model: interaction of genetic vulnerability and consequences of maternal separation. *Behav. Brain Res.* **177** 142–149

Pesold C, Impagnatiello F, Pisu MG, Uzunov DP, Costa E, Guidotti A and Caruncho HJ 1998 Reelin is preferentially expressed in neurons synthesizing gamma-aminobutyric acid in cortex and hippocampus of adult rats. *Proc. Natl. Acad. Sci. USA* **95** 3221–3226

Pfaffl MW 2001 A new mathematical model for relative quantification in real-time RT-PCR *Nucleic Acids Res.* **29** e45

Qin L, Tu W, Sun X, Zhang J, Chen Y and Zhao H 2011 Retardation of neurobehavioral development and reelin down-regulation regulated by further DNA methylation in the hippocampus of the

rat pups are associated with maternal deprivation. *Behav. Brain Res.* **217** 142–147

Qiu S and Weeber EJ 2007 Reelin signaling facilitates maturation of CA1 glutamatergic synapses. *J. Neurophysiol.* **97** 2312–2321

Qiu S, Zhao LF, Korwek KM and Weeber EJ 2006 Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *J. Neurosci.* **265** 12943–12955

Roskoden T, Otten U and Schwegler H 2004 Early postnatal corticosterone administration regulates neurotrophins and their receptors in septum and hippocampus of the rat. *Exp. Brain Res.* **154** 183–191

Rosztoczy A, Fioramonti J, Jarmay K, Barreau F, Wittmann T and Bueno L 2003 Influence of sex and experimental protocol on the effect of maternal deprivation on rectal sensitivity to distension in the adult rat. *Neurogastroenterol. Motil.* **15** 679–686

Sapolsky RM 2000 The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. *Biol. Psychiatry* **48** 755–765

Schore AN 2000 Attachment and the regulation of the right brain. *Attach Hum. Dev.* **2** 23–47

Seckl JR and Meaney MJ 2004 Glucocorticoid programming. *Ann. NY Acad. Sci.* **1032** 63–84

Soda T, Nakashima R, Watanabe D, Nakajima K, Pastan I and Nakanishi S 2003 Segregation and coactivation of developing neocortical layer 1 neurons. *J. Neurosci.* **23** 6272–6279

Tronick E, Weinberg M 1997 Depressed mothers and infants: failure to form dyadic states of consciousness; in *Postpartum depression and child development* (eds) L Murray and PJ Cooper (NY: Guilford) pp 54–81

Vermetten E and Bremner JD 2002 Circuits and systems in stress. I Preclinical studies. *Depress Anxiety* **15** 126–147

Weaver IC, Grant RJ and Meaney MJ 2002 Maternal behavior regulates long-term hippocampal expression of BAX and apoptosis in the offspring. *J. Neurochem.* **82** 998–1002

Weaver IC, Meaney MJ and Szyf M 2006 Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc. Natl. Acad. Sci. USA* **103** 3480–3485

Zhao H, Xu H, Xu X and Young D 2007 Predatory stress induces hippocampal cell death by apoptosis in rats. *Neurosci. Lett.* **421** 115–120

MS received 07 December 2011; accepted 06 November 2012

Corresponding editor: VIDITA A VAIDYA