Decreased hippocampal mineralocorticoid:glucocorticoid receptor ratio is associated with low birth weight in female cynomolgus macaque neonates

Shirlene X Ong[1], Keefe Chng[1], Michael J Meaney[1,2] and Jan P Buschdorf[1]

[1]Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Brenner Centre for Molecular Medicine, 30 Medical Drive, Singapore 117609, Singapore
[2]Canadian Neuroepigenetics Network, Douglas Mental Health University Institute, McGill University, 6875 LaSalle Boulevard, Montreal, Quebec H4H 1R3, Canada

Correspondence should be addressed to J P Buschdorf
Email
janpaul@sics.a-star.edu.sg

Journal of Molecular Endocrinology

Abstract

During pregnancy, glucocorticoids transfer environmental signals to the growing brain and its associated neuroendocrine system to modulate their maturation and function during adolescence and adulthood. Increased *in utero* exposure to glucocorticoids is associated with impaired fetal growth resulting in low birth weight (LBW) and compromised neural development. The underlying molecular changes affecting brain development, however, are largely unknown. Here, we compared the relative mRNA expression of genes directly involved in glucocorticoid signaling in the hippocampus, amygdala, and cortex of female non-human primate neonates (*Macaca fascicularis*) of naturally occurring normal birth weight and LBW. We focused on the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) genes as well as that for 11β-hydroxysteroid dehydrogenase type 1 (*11β-HSD1*) and found a significantly decreased MR:GR mRNA ratio in the hippocampus and lower expression of *11β-HSD1* in the amygdala associated with LBW. The MR:GR mRNA ratio in the amygdala and cortex was not associated with birth weight, reflecting tissue-specific effects. Protein quantification in the hippocampus confirmed our finding of a decreased hippocampal MR:GR ratio. Our data suggest that the MR:GR ratio in the hippocampus and the expression of *11β-HSD1* in the amygdala are associated with intrauterine growth restriction in non-human primates during early perinatal development.

Key Words
- glucocorticoid receptor
- cynomolgus macaque
- low birth weight
- MR:GR ratio

Journal of Molecular Endocrinology
(2013) **51**, 59–67

Introduction

The quality of fetal growth predicts that of health over the life span, with a substantial effect on brain-based disorders such as attention-deficit hyperactivity disorder (Bhutta *et al*. 2002) and depression (Thompson *et al*. 2001, Alati *et al*. 2007). Hippocampal structure and function associate with cognitive and mood disorders, and low birth weight (LBW) is associated with reduced hippocampal volume (Buss *et al*. 2007, de Bie *et al*. 2010). While human small for gestational age infants show reduced brain weight and cell number in the brain compared with normal birth weight

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

(NBW) controls (de Bie *et al*. 2010), the hippocampus appears particularly vulnerable. Studies with appropriate animal models show that intrauterine growth restriction (IUGR) associates with reduced hippocampal volume (Mallard *et al*. 2000, Lister *et al*. 2005).

There is considerable evidence in humans and other species that impaired fetal growth is linked to increased *in utero* glucocorticoid exposure (Reinisch *et al*. 1978, French *et al*. 1999, Bloom *et al*. 2001, Nyirenda *et al*. 2001, Meaney *et al*. 2007). Cord blood samples obtained from LBW human babies reveal significant elevations in both corticotrophin-releasing factor (CRF) and cortisol (Goland *et al*. 1993). Interestingly, maternal stress, protein deprivation, tobacco, and alcohol predict impaired fetal growth and increase maternal adrenal glucocorticoid release, which increases placental CRF expression; hence the relationship between maternal and fetal glucocorticoids – placental CRF in IUGR babies (Meaney *et al*. 2007). Moreover, levels of placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), a placental barrier enzyme that inactivates cortisol, are reduced in LBW babies (Seckl *et al*. 1999, McTernan *et al*. 2002). Glucocorticoid administration to pregnant female rodents reduces birth weight (Nyirenda *et al*. 2001). Likewise, increased fetal glucocorticoid exposure appears to mediate the effects of conditions that associate with impaired fetal growth, such as malnutrition. Protein malnutrition increases fetal glucocorticoid levels in mice as well as in sheep (Bloomfield *et al*. 2003, Cottrell *et al*. 2012) and maternal adrenalectomy blocks the effects on fetal growth.

Glucocorticoids are important for normal intrauterine and postnatal brain development, regulating processes such as glial proliferation and myelinogenesis, remodeling axonal and dentritic spines, and affecting cell survival (Meyer 1983, Lupien *et al*. 2009). Nevertheless, treatments that elevate glucocorticoid levels during late pregnancy compromise brain development and correlate with poorer cognitive performance in humans (Damsted *et al*. 2011). A reduction in hippocampal volume was found in primates exposed to exogenous glucocorticoids before birth (Uno *et al*. 1994). These findings suggest that the increased *in utero* exposure to glucocorticoids that defines the endocrine conditions associated with impaired fetal growth compromises neural development. While fetal growth restriction appears to be associated with increased glucocorticoid exposure, little is known about the expression of corticosteroid receptors in late fetal or early postnatal development. Glucocorticoid receptor (GR) expression is dynamically regulated over perinatal development in a highly tissue-specific manner (Kalinyak *et al*. 1989, Bohn *et al*. 1994). It is critical to assess corticosteroid receptor levels to evaluate potential tissue sensitivity to corticosteroids as a function of fetal growth. In this study, we compared the relative mRNA expression of genes directly involved in glucocorticoid signaling in the hippocampus, amygdala, and cortex of non-human primate neonates (cynomolgus macaque) of naturally occurring NBW and LBW, focusing on the glucocorticoid receptor and mineralocorticoid receptor genes as well as that for 11β-HSD1, which converts inactive corticosteroids to cortisol and associates with increased corticosteroid action (Wyrwoll *et al*. 2011). We report that the MR:GR mRNA ratio is decreased in the ratio of the hippocampus of LBW animals. A similar decrease in the corresponding proteins was observed in hippocampal samples analyzed by western blotting. *11β-HSD1* mRNA showed reduced expression in the amygdala of the same group of animals. The results point to an association of a decreased MR:GR ratio in the hippocampus and reduced expression of *11β-HSD1* mRNA in the amygdala with fetal growth restriction during perinatal development.

Materials and methods

Animals and collection of brain tissue

The animals were bred and killed at the Nafovanny facility in Vietnam. All animal procedures were approved by Nafovanny. Oversight of Nafovanny is provided by the Vietnamese Ministry of Forestry. Our studies were approved by the Singapore Health Institutional Animal Care and Use Committee and performed in accordance with the Guidelines on the Care and Use of Animals for Scientific Purposes set by the National Advisory Committee for Laboratory Animal Research (NACLAR, http://www.ava.gov.sg/AnimalsPetSector/CareAndUse-AnimalsForScientificPurp/) of Singapore, which is based on the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, NHMRC, Australia, as well as the Guide for the Care and Use of Laboratory Animals by the National Academy of Sciences, USA (courtesy of the National Academies Press, Washington, DC, USA), and The Good Practice Guide for the Use of Animals in Research, Testing and Teaching, National Animal Ethics Advisory Committee, New Zealand. Sixty-four pregnant cynomolgus macaque dams (*Macaca fascicularis*), sired naturally, were monitored before delivery at the Vietnam Primate Breeding and Development Corporation. These 64 pregnant dams gave birth to 65 male and female neonates (original cohort of neonates; Table 1).

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

Table 1 Cohort and sample details of all animals (*Macaca fascicularis*)

	Original cohort	NBW group	LBW group
Number of females/males	33/32		
Number of still births	4		
Number of singletons/twins	63/2		
Birth weight (g) (and age (days)) of each animal			
1		358 (2.5)	
2		371 (5.6)	
3		358 (3.6)	
4		368 (7.3)	
5		351 (4.6)	
6			316 (4.5)
7			323 (3.6)
8			317 (6.5)
9			317 (4.6)
10			314 (4.5)
11			326 (6.6)
Mean ± S.D. birth weight (g)	358 ± 40.7	361 ± 8.2	319 ± 4.2
Median (and range of) birth weight[a] (g)	351 (299–480)		
Birth weight percentile of the original female cohort		50–65th	4–27th
Mean age (days)			
Hippocampus		4.7 ($n=5$)	5.1 ($n=6$)
Amygdala		4.8 ($n=4$)	5.3 ($n=5$)
Cortex		4.1 ($n=4$)	5.1 ($n=5$)

NBW, normal birth weight; LBW, low birth weight.
[a]Median birth weight of females (excluding still births and twins) in grams.

The normative birth weight range was assessed and 11 female neonates were selected based on their birth weights to comprise two groups (see Table 1 for details): i) LBW group with $n=6$ and a birth weight range of 316–326 g (4–27th birth weight percentile of the original female cohort) and ii) NBW group with $n=5$ and a birth weight range of 358–371 g (50–65th birth weight percentile of the original female cohort). The average age of the NBW group was 4.7 days and that of the LBW group was 5.1 days. All neonates were sedated between 0920 and 1115 h with an i.m. injection of ketamine–HCl (15 mg/kg) and exsanguinated under anesthesia. Whole brains were collected and stored at −80 °C.

Tissue processing

Coronal sections from the right hippocampus and the right amygdala (including adjacent cortical tissue) were prepared at an interval of two sections of 300 and 20 μm respectively by cryosectioning and thaw-mounted on poly-lysine-coated slides. Slides were stored at −80 °C until further processing for Nissl staining and RNA extraction. Nissl staining facilitated the identification of the regions of interest, e.g. right anterior hippocampus, cortex, and right amygdala. We defined the anterior hippocampus as the hippocampal part that is present in the coordinates A9.6 to A5.6 of the stereotaxic brain atlas by Szabo & Cowan (1984) (Fig. 1A). The lateral geniculate nucleus was used as a landmark (Fig. 1A). The cortical tissue (containing the parietal area-associated area of the superior temporal sulcus and the intraparietal sulcus-associated area in the superior temporal sulcus) was taken from the same section as the hippocampus. The amygdala was identified by the presence of its paralaminar nucleus and/or its characteristic shape and location anterior to the hippocampus (Fig. 1B). A standard protocol was used for Nissl staining of 20 μm brain sections. Pictures were taken with an Axio Observer Z1 microscope (Zeiss, Oberkochen, Baden-Wuerttemberg, Germany) and stitched with TissueFAXS suite version 3 Software (TissueGnostics, Vienna, Austria).

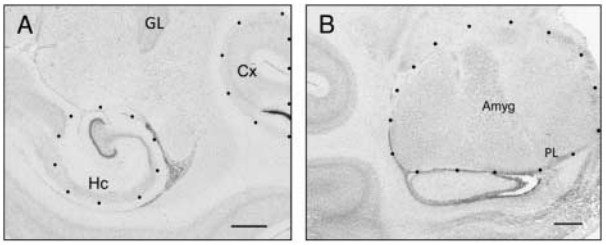

Figure 1
Representative Nissl stainings of the hippocampus (A) and amygdala (B) of female cynomolgus macaque neonates. Black dots demarcate the regions (hippocampus, cortex, and amygdala) analyzed in our study; the scale bars represent 1 mm. Amyg, amygdala; Cx, cortex; GL, dorsal lateral geniculate nucleus; Hc, hippocampus; PL, paralaminar nucleus of amygdala.

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

RNA extraction and cDNA synthesis

Areas used for RNA extraction from the hippocampus, amygdala, and cortex are indicated in Fig. 1A and B. Total RNA was isolated from a single 300 μm section, using the All Prep DNA/RNA Micro Kit (Qiagen) following the manufacturer's protocol. RNA quantity was measured spectrophotometrically (Nanophotometer, Implen, Munich, Germany). First-strand synthesis of cDNA was carried out with oligo-dT primer using the Transcriptor First Strand cDNA synthesis kit (Roche). cDNAs were tested for genomic contamination with a genomic contamination primer assay (Qiagen).

Quantitative real-time PCR

Quantitative real-time PCR was performed on an LC480 II Real-Time PCR instrument with Light Cycler 480 SYBR Green I Mastermix (Roche). All primers were designed with published sequences of *Macaca mulatta* and are listed in Supplementary Table 1, see section on supplementary data given at the end of this article. Pre-designed primers were purchased from Qiagen. Primer pairs for the amplification of different first exons of the *NR3C1* gene were designed with the forward primer targeting a specific first exon while the target sequence for the reverse primer was in exon 2. The specificity of these primers was confirmed by cloning and sequencing of the PCR products. Primer efficiencies were determined with CAmpER Software (www.cebitec.uni-bielefeld.de) using the DART method (Peirson *et al.* 2003). Quantification was performed with the Advanced Relative Quantification application of the Light Cycler 480 Software 1.5 (Roche). Samples were analyzed in triplicates and normalized to the expression of β2-microglobulin. Values are expressed in arbitrary units. Pilot assays showed significant differences in GAPDH expression as a function of birth weight, but no effect on β2-microglobulin.

Protein extraction and western blotting

Hippocampal proteins were isolated from two 300 μm sections from an area as indicated in Fig. 1A. Tissue was lysed in RIPA buffer (containing 1% (v/v) Triton X-100 and 1% (w/v) sodium deoxycholate) by vortexing and sonication with a Bioruptor (Diagenode, Liège, Belgium)). Protein concentration was determined with the Dc Protein Assay Kit (Bio-Rad). Twenty micrograms of proteins were analyzed by western blotting with the following antibodies: GFAP (Millipore), GR (Millipore), neurofilament-L (Cell Signaling Technology), and MR (Novus Biologicals). β-Tubulin and β-actin antibodies (both from Sigma) were used to demonstrate equal loading. Quantification of protein bands was carried out with the Odyssey Infrared Imaging System and Odyssey Software version 2.1 (LI-COR, Lincoln, NE, USA) according to the manufacturer's instruction. For the calculation of the MR:GR protein ratio, we added all the values for the different GR isoforms detected in a given sample.

Statistical analysis

Data are presented as means ± S.E.M. using GraphPad Prism 5 Software, Version 5.04 (GraphPad Software, San Diego, CA, USA). Student's *t*-tests were used to statistically compare differences in gene expression between samples from LBW and NBW neonates with a significance cutoff value of $P<0.05$. We removed one outlying value (animal 5 from the NBW group) from the hippocampal MR:GR mRNA ratios that was more than 4 S.D.s from the mean. There were no outlying values in any of the remaining analyses.

Results

The aim of this study was to characterize gene expression differences of candidate genes associated with glucocorticoid signaling in different brain regions early in development as a function of birth weight across the normal range in non-manipulated cynomolgus macaques. From an original cohort of 65 male and female neonates, we selected female neonates to form two groups (see Table 1 for details): a NBW group ($n=5$, birth weight range of 351–371 g, 50–65th birth weight percentile of the original female cohort) and a LBW group ($n=6$, birth weight range of 316–326 g, 4–27th birth weight percentile of the original female cohort). The respective mean birth weights differed significantly ($t_9=10.86$, $P<0.001$; mean 361.2 g, S.E.M. 3.7 g for NBW vs mean 318.8 g, S.E.M. 1.9 g for LBW). The brain regions included in our study were the anterior hippocampus, cortex (adjacent to the hippocampus), and the amygdala, all dissected from the right temporal lobe (Fig. 1A and B). The hippocampi of all 11 samples were included in our study ($n=5$ for NBW group and $n=6$ for LBW group). Owing to impaired integrity of some of the brain samples, group sizes for the amygdala and cortex were $n=4$ for NBW and $n=5$ for LBW neonates. As a result, the mean age difference between the two groups varied from 0.4 to 1 day (see Table 1 for details). We analyzed the relative expression level of genes encoding the GR (*NR3C1*), the MR (*NR3C2*) and 11β-HSD1 (*11β-HSD1*). Although we did not detect any statistically significant differences for the three genes

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

examined individually (Fig. 2A, B and C), the ratio of MR:GR mRNA expression was significantly decreased in hippocampal samples from LBW compared with NBW animals (t_8=5.147, P=0.001; Fig. 2D).

The human GR gene contains at least ten alternative non-coding first exons (Turner *et al.* 2006, Presul *et al.* 2007). We analyzed the usage of the following first exons: 1A, 1B, 1C3, 1D, 1E, 1F, 1G, and 1H. Expression of exons 1A, 1D, 1E, and 1G was not detected. No significant differences in the transcript levels for exons 1B, 1C3, 1H, and 1F were found as a function of birth weight (data not shown).

In light of the difference of the hippocampal GR:MR ratio, we estimated the cellular composition in hippocampal samples to ensure that differences in corticosteroid receptor mRNA levels were not associated with differences in the relative inclusion of glial and neuronal cells in our assays. We thus examined hippocampal expression of the neuronal marker protein neurofilament-L and the glial marker protein GFAP by western blotting. Comparable expression of both proteins was detected across all samples (Fig. 3A), suggesting that up-regulation of GR expression in LBW hippocampi is not associated with variation in neuron and glial cell numbers.

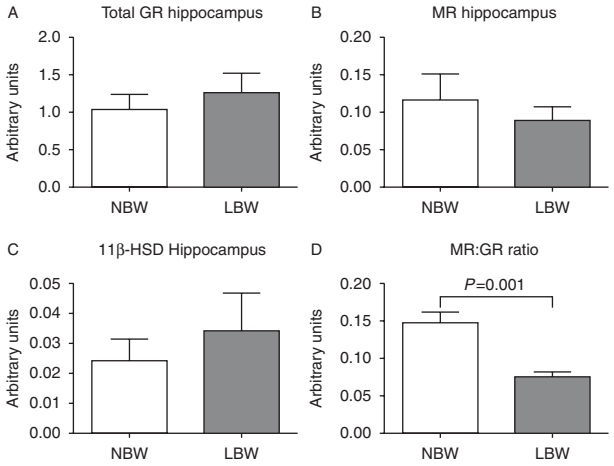

Figure 2
Normalized expression values for total GR (A), MR (B), and 11β-HSD (C) mRNAs in the hippocampus of NBW and LBW cynomolgus macaque neonates. In addition, (D) presents the hippocampal MR:GR ratio calculated with the corresponding expression values. Data are normalized to the expression of β2-microglobulin and expressed as arbitrary units. Only statistically significant differences are indicated. *t*-test was used for comparison. Bars show mean values and S.E.M. GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase type 1; MR, mineralocorticoid receptor; LBW, low birth weight; NBW, normal birth weight.

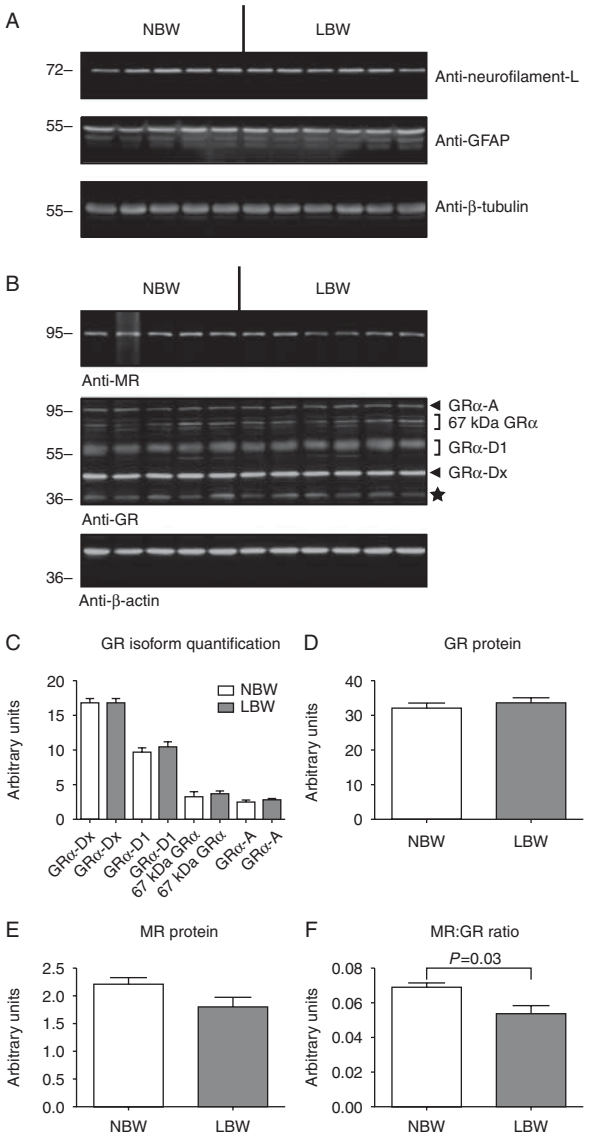

Figure 3
Analysis of neurofilament-L and GFAP expression (A) and MR as well as GR expression (B) in the hippocampus of NBW and LBW cynomolgus macaque neonates by western blotting. Hippocampal protein lysates were prepared and analyzed by western blotting with the indicated antibodies. In (B), GR isoforms are indicated to the right of the western blot. Arrowheads and brackets specify the region for protein quantification of the respective isoform. An additional, uncharacterized band is labeled with a star. Quantification of GR isoforms (C), GR (D; sum of all isoforms quantified), and MR (E). In addition, (D) presents the hippocampal MR:GR ratio calculated with the corresponding values. Bars show mean values and S.E.M. GR, glucocorticoid receptor; MR, mineralocorticoid receptor; LBW, low birth weight; NBW, normal birth weight.

Additional western blotting revealed the expression pattern for GR protein in the hippocampus of NBW and LBW neonates (Fig. 3B). The GR protein is expressed in several N-terminal variants. The bands included are

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

indicated in Fig. 3B and quantified in Fig. 3C. Our blot contained four of the five protein isoforms described in humans (Sinclair *et al.* 2011): quantification of these isoforms revealed a higher expression of isoforms GRα-D1 and GRα-Dx, with no group differences in any of the single isoforms (data not shown and Fig. 3C). The latter result is consistent with the quantification of the GR mRNA in the hippocampus of the NBW and LBW groups (Fig. 2A). Our blot did not contain proteins smaller than circa 30 kDa; hence, we were unable to detect the fifth GR isoform of about 25 kDa (Sinclair *et al.* 2011). The blot was re-probed with an anti-MR antibody to detect MR expression in the hippocampus (Fig. 3B). As with the MR mRNA expression, quantification and statistical analysis did not reveal any difference in protein level between the two birth weight groups (Fig. 3E). However, calculation of the MR:GR protein ratio (see Material and methods section for details) showed a significantly reduced ratio in the LBW group compared with the control group ($t_9=2.636$, $P=0.03$; Fig. 3F). This result is in agreement with the reduced MR:GR mRNA ratio presented in Fig. 2D.

Expression of the GR, the MR, and *11β-HSD1* in the NBW and LBW groups was analyzed in the amygdala and cortex. There was significantly lower expression of *11β-HSD1* in the amygdala of the LBW group ($t_7=3.03$, $P=0.02$; Fig. 4C). No significant changes were detected for the other genes and tissues tested (Fig. 4A, B, D, E and F), although the difference in the MR mRNA expression in the cortex approached significance ($t_7=2.019$, $P=0.08$; Fig. 4E). Likewise, there was no statistically significant change in the GR:MR mRNA ratio in the amygdala and cortex respectively (data not shown).

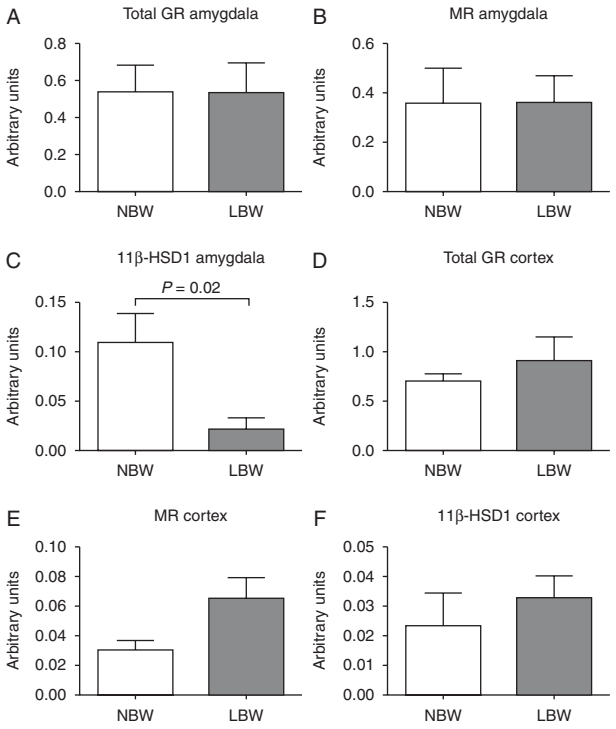

Figure 4
Normalized expression values for total GR, MR, and 11β-HSD mRNAs in the amygdala (A, B and C) and cortex (D, E and F) of NBW and LBW cynomolgus macaque neonates. Expression data are normalized to the expression of β2-microglobulin and expressed as arbitrary units. Only statistically significant differences are indicated. *t*-test was used for comparison. Bars show mean values and S.E.M. GR, glucocorticoid receptor; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; MR, mineralocorticoid receptor; LBW, low birth weight; NBW, normal birth weight.

Discussion

Fetal growth restriction associates with increased fetal exposure to glucocorticoids through either direct activity of the adrenal or indirectly through reduced expression of 11β-HSD2, a placental barrier enzyme that metabolizes cortisol (or corticosterone in rodents) to biologically inactive corticosteroids (Seckl *et al.* 1999, Meaney *et al.* 2007). Maternal stress or exposure to elevated levels of glucocorticoids compromises hippocampal development in non-human primates (Uno *et al.* 1989, Coe *et al.* 2003) and is a candidate mechanism for the compromised neural development associated with IGUR states. However, the status of the relevant corticosteroid signaling systems in the developing hippocampus was unknown. The results of our studies with a cynomolgus macaque model of fetal growth suggest that LBW associates with decreased hippocampal GR:MR mRNA and protein ratios, with levels of GR and MR protein that are at least comparable with animals of NBW. These findings reveal that LBW associates with sustained hippocampal sensitivity to glucocorticoids.

The MR/GR balance hypothesis (Joëls *et al.* 2007) suggests that the relative activity of the MR and the GR (and other mediators of the stress response) determine the sensitivity and response of the brain to glucocorticoid signaling and stress in general. A normal ratio of these receptors promotes health, homeostasis, and adaptation. The MR rapidly enhances the excitability of hippocampal neurons through a non-genomic action in response to elevated glucocorticoid levels (Karst *et al.* 2005, Qiu *et al.* 2010) indicating a role for this receptor in the acute behavioral response to stress. Downregulation of the hypothalamic–pituitary–adrenal (HPA) axis, the principle mediator of the stress response, is achieved via a negative feedback mediated by activation of GRs in the hippocampus and prefrontal cortex. Recently, the MR:GR ratio

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

was found to control activity of the HPA axis under stressed but not basal conditions in mice (Harris *et al.* 2012). An imbalance of these two receptors early in life as detected in the hippocampus of LBW neonates in our study may alter the glucocorticoid sensitivity of this brain structure. In contrast to the hippocampus, we did not detect a difference in the GR:MR mRNA ratio in the amygdala. However, reduced expression of 11β-HSD1, an enzyme that converts inactive corticosteroids to cortisol, in the amygdala of LBW animals might also change its glucocorticoid sensitivity.

Studies of Dean & Matthews (1999) and Sloboda *et al.* (2008) demonstrated the impact of synthetic glucocorticoid administration on the expression of fetal MR and GR: repeated maternal dexa/betamethasone injection enhanced fetal expression of both receptors in the hippocampus. By contrast, fetal corticosterone exposure or prenatal stress stably decreases MR as well as GR expression and increases basal corticosterone levels in adult rats (McCormick *et al.* 1995, Levitt *et al.* 1996, Welberg *et al.* 2001). Likewise, early-life stress in the common marmoset leads to long-term mild hippocampal decreases in GR as well as MR expression at adolescence (Arabadzisz *et al.* 2010). These studies suggest a dynamic regulation of corticosteroid receptor transcription over development by glucocorticoids induced by environmental factors like stress or food restriction. In our study, we did not detect a significant change in hippocampal corticosteroid receptor expression between the two birth weight groups. This might be explained by the fact that we used non-manipulated animals where changes might be more subtle compared with experimental conditions. Nevertheless, the change in the MR:GR ratio in the LBW group was highly significant (and not detected in the two other brain structures analyzed), suggesting indeed a dynamic regulation by environmental factors. Further studies are necessary to clarify whether the alterations in hippocampal MR:GR ratio in the hippocampus of LBW cynomolgus macaques are sustained into later life.

Our findings suggest differential relative hippocampal expression of the GR and MR as a function of birth weight. Importantly, activation of these receptors leads to contrasting effects on neural function (Joëls *et al.* 2007). MR activation maintains neuronal excitability and promotes neurogenesis (Gass *et al.* 2000, Joëls *et al.* 2007); MR knockout mice show profound reductions in hippocampal development. By contrast, activation of the GR dampens neuronal excitability and may inhibit neurogenesis and generally compromises synaptic plasticity (McEwen 1999). While a matter of some speculation, the results presented here suggest the potential for differential MR/GR activation in the hippocampus as a function of fetal growth. Similarly, downregulation of 11β-HSD1 in the amygdala in the LBW group seems to be a result of impaired fetal growth. Like the hippocampus, the volume of the amygdala increases postnatally (Lupien *et al.* 2009). We hypothesize that the changed GR:MR ratio in the hippocampus and the lower expression of 11β-HSD1 in the amygdala are manifestations of an altered developmental trajectory for the LBW neonates that might lead to impaired brain function and changes in the HPA axis adjustment.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JME-12-0218.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was supported by the Agency of Science, Technology and Research (A*STAR), Singapore.

Acknowledgements

The authors thank Dr Makoto Yawata for his assistance in taking pictures. They also thank Drs Joanna Holbrook, Walter Stünkel, and Judy Sng for critically reading the manuscript.

References

Alati R, Lawlor DA, Mamun AA, Williams GM, Najman JM, O'Callaghan M & Bor W 2007 Is there a fetal origin of depression? Evidence from the Mater University Study of Pregnancy and its outcomes *American Journal of Epidemiology* **165** 575–5782. (doi:10.1093/aje/kwk036)

Arabadzisz D, Diaz-Heijtz R, Knuesel I, Weber E, Pilloud S, Dettling AC, Feldon J, Law AJ, Harrison PJ & Pryce CR 2010 Primate early life stress leads to long-term mild hippocampal decreases in corticosteroid receptor expression. *Biological Psychiatry* **67** 1106–1109. (doi:10.1016/j.biopsych.2009.12.016)

Bhutta AT, Cleves MA, Casey PH, Cradock MM & Anand KJS 2002 Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *Journal of the American Medical Association* **288** 728–737. (doi:10.1001/jama.288.6.728)

de Bie HM, Oostrom KJ & Delemarre-van de Waal HA 2010 Brain development, intelligence and cognitive outcome in children born small for gestational age. *Hormone Research in Paediatrics* **73** 6–14. (doi:10.1159/000271911)

Bloom SL, Sheffield JS, McIntire DD & Leveno KJ 2001 Antenatal dexamethasone and decreased birth weight. *Obstetrics and Gynecology* **97** 485–490. (doi:10.1016/S0029-7844(00)01206-0)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

Bloomfield FH, Oliver MH, Hawkins P, Campbell M, Phillips DJ, Gluckman PD, Challis JR & Harding JE 2003 A periconceptional nutritional origin for noninfectious preterm birth. *Science* **300** 606. (doi:10.1126/science.1080803)

Bohn MC, Dean D, Hussain S & Giuliano R 1994 Development of mRNAs for glucocorticoid and mineralocorticoid receptors in rat hippocampus. *Brain Research. Developmental Brain Research* **77** 157–162. (doi:10.1016/0165-3806(94)90192-9)

Buss C, Lord C, Wadiwalla M, Hellhammer DH, Lupien SJ, Meaney MJ & Pruessner JC 2007 Maternal care modulates the relationship between prenatal risk and hippocampal volume in women but not in men. *Journal of Neuroscience* **27** 2592–2595. (doi:10.1523/JNEUROSCI.3252-06.2007)

Coe CL, Kramer M, Czéh B, Gould E, Reeves AJ, Kirschbaum C & Fuchs E 2003 Prenatal stress diminishes neurogenesis in the dentate gyrus of juvenile rhesus monkeys. *Biological Psychiatry* **54** 1025–1034. (doi:10.1016/S0006-3223(03)00698-X)

Cottrell EC, Holmes MC, Livingstone DE, Kenyon CJ & Seckl JR 2012 Reconciling the nutritional and glucocorticoid hypotheses of fetal programming. *FASEB Journal* **26** 1866–1874. (doi:10.1096/fj.12-203489)

Damsted SK, Born AP, Paulson OB & Uldall P 2011 Exogenous glucocorticoids and adverse cerebral effects in children. *European Journal of Paediatric Neurology* **15** 465–477. (doi:10.1016/j.ejpn.2011.05.002)

Dean F & Matthews SG 1999 Maternal dexamethasone treatment in late gestation alters glucocorticoid and mineralocorticoid receptor mRNA in the fetal guinea pig brain. *Brain Research* **846** 253–259. (doi:10.1016/S0006-8993(99)02064-8)

French NP, Hagan R, Evans SF, Godfrey M & Newnham JP 1999 Repeated antenatal corticosteroids: size at birth and subsequent development. *American Journal of Obstetrics and Gynecology* **180** 114–121. (doi:10.1016/S0002-9378(99)70160-2)

Gass P, Kretz O, Wolfer DP, Berger S, Tronche F, Reichardt HM, Kellendonk C, Lipp HP, Schmid W & Schütz G 2000 Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Reports* **1** 447–451. (doi:10.1093/embo-reports/kvd088)

Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI & Tropper PJ 1993 Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. *Journal of Clinical Endocrinology and Metabolism* **77** 1174–1179. (doi:10.1210/jc.77.5.1174)

Harris AP, Holmes MC, de Kloet ER, Chapman KE & Seckl JR 2012 Mineralocorticoid and glucocorticoid receptor balance in control of HPA axis and behaviour. *Psychoneuroendocrinology* **38** 648–658. (doi:10.1016/j.psyneuen.2012.08.007)

Joëls M, Karst H, DeRijk R & de Kloet ER 2007 The coming out of the brain mineralocorticoid receptor. *Trends in Neurosciences* **3** 1–7. (doi:10.1016/j.tins.2007.10.005)

Kalinyak JE, Griffin CA, Hamilton RW, Bradshaw JG, Perlman AJ & Hoffman AR 1989 Developmental and hormonal regulation of glucocorticoid receptor messenger RNA in the rat. *Journal of Clinical Investigation* **84** 1843–1848. (doi:10.1172/JCI114370)

Karst H, Berger S, Turiault M, Tronche F, Schütz G & Joëls M 2005 Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. *PNAS* **102** 19204–19207. (doi:10.1073/pnas.0507572102)

Levitt NS, Lindsay RS, Holmes MC & Seckl JR 1996 Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* **64** 412–418. (doi:10.1159/000127146)

Lister JP, Blatt GJ, DeBassio WA, Kemper TL, Tonkiss J, Galler JR & Rosene DL 2005 Effect of prenatal protein malnutrition on numbers of neurons in the principal cell layers of the adult rat hippocampal formation. *Hippocampus* **15** 393–403. (doi:10.1002/hipo.20065)

Lupien SJ, McEwen BS, Gunnar MR & Heim C 2009 Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews. Neuroscience* **10** 434–445. (doi:10.1038/nrn2639)

Mallard C, Loeliger M, Copolov D & Rees S 2000 Reduced number of neurons in the hippocampus and the cerebellum in the postnatal guinea-pig following intrauterine growth-restriction. *Neuroscience* **100** 327–333. (doi:10.1016/S0306-4522(00)00271-2)

McCormick CM, Smythe JW, Sharma S & Meaney MJ 1995 Sex-specific effects of prenatal stress on hypothalamic–pituitary–adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Brain Research. Developmental Brain Research* **84** 55–61. (doi:10.1016/0165-3806(94)00153-Q)

McEwen BS 1999 Stress and hippocampal plasticity. *Annual Review of Neuroscience* **22** 105–122. (doi:10.1146/annurev.neuro.22.1.105)

McTernan CL, Draper N, Nicholson H, Chalder SM, Driver P, Hewison M, Kilby MD & Stewart PM 2002 Reduced placental 11β-hydroxysteroid dehydrogenase type 2 mRNA levels in human pregnancies complicated by intrauterine growth restriction: an analysis of possible mechanisms. *Journal of Clinical Endocrinology and Metabolism* **86** 4979–4983. (doi:10.1210/jc.86.10.4979)

Meaney MJ, Szyf M & Seckl JR 2007 Epigenetic mechanisms of perinatal programming of hypothalamic–pituitary–adrenal function and health. *Trends in Molecular Medicine* **13** 269–277. (doi:10.1016/j.molmed.2007.05.003)

Meyer JS 1983 Early adrenalectomy stimulates subsequent growth and development of the rat brain. *Experimental Neurology* **82** 432–446. (doi:10.1016/0014-4886(83)90415-6)

Nyirenda MJ, Welberg LA & Seckl JR 2001 Programming hyperglycaemia in the rat through prenatal exposure to glucocorticoids – fetal effect or maternal influence? *Journal of Endocrinology* **170** 653–660. (doi:10.1677/joe.0.1700653)

Peirson SN, Butler JN & Foster RG 2003 Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. *Nucleic Acids Research* **31** e73. (doi:10.1093/nar/gng073)

Presul E, Schmidt S, Kofler R & Helmberg A 2007 Identification, tissue expression, and glucocorticoid responsiveness of alternative first exons of the human glucocorticoid receptor. *Journal of Molecular Endocrinology* **38** 79–90. (doi:10.1677/jme.1.02183)

Qiu S, Champagne DL, Peters M, Catania EH, Weeber EJ, Levitt P & Pimenta AF 2010 Loss of limbic system-associated membrane protein leads to reduced hippocampal mineralocorticoid receptor expression, impaired synaptic plasticity, and spatial memory deficit. *Biological Psychiatry* **68** 197–204. (doi:10.1016/j.biopsych.2010.02.013)

Reinisch JM, Simon NG, Karow WG & Gandelman R 1978 Prenatal exposure to prednisone in humans and animals retards intrauterine growth. *Science* **202** 436–438. (doi:10.1126/science.705336)

Seckl JR, Nyirenda MJ, Walker BR & Chapman KE 1999 Glucocorticoids and fetal programming. *Biochemical Society Transactions* **27** 74–78.

Sinclair D, Webster MJ, Wong J & Weickert CS 2011 Dynamic molecular and anatomical changes in the glucocorticoid receptor in human cortical development. *Molecular Psychiatry* **16** 504–515. (doi:10.1038/mp.2010.28)

Sloboda DM, Moss TJ, Li S, Matthews SG, Challis JR & Newnham JP 2008 Expression of glucocorticoid receptor, mineralocorticoid receptor, and 11β-hydroxysteroid dehydrogenase 1 and 2 in the fetal and postnatal ovine hippocampus: ontogeny and effects of prenatal glucocorticoid exposure. *Journal of Endocrinology* **197** 213–220. (doi:10.1677/JOE-07-0375)

Szabo J & Cowan WM 1984 A stereotaxic atlas of the brain of the cynomolgus monkey (*Macaca fascicularis*). *Journal of Comparative Neurology* **222** 265–300. (doi:10.1002/cne.902220208)

Thompson C, Syddall H, Rodin I, Osmond C & Barker DJ 2001 Birth weight and the risk of depressive disorder in late life. *British Journal of Psychiatry* **179** 450–455. (doi:10.1192/bjp.179.5.450)

Turner JD, Schote AB, Macedo JA, Pelascini LP & Muller CP 2006 Tissue specific glucocorticoid receptor expression, a role for alternative first

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.

exon usage? *Biochemical Pharmacology* **72** 1529–1537. (doi:10.1016/j.bcp.2006.07.005)

Uno H, Tarara R, Else JG, Suleman MA & Sapolsky RM 1989 Hippocampal damage associated with prolonged and fatal stress in primates. *Journal of Neuroscience* **9** 1705–1711.

Uno H, Eisele S, Sakai A, Shelton S, Baker E, DeJesus O & Holden J 1994 Neurotoxicity of glucocorticoids in the primate brain. *Hormones and Behavior* **28** 336–348. (doi:10.1006/hbeh.1994.1030)

Welberg LA, Seckl JR & Holmes MC 2001 Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience* **104** 71–79. (doi:10.1016/S0306-4522(01)00065-3)

Wyrwoll CS, Holmes MC & Seckl JR 2011 11β-Hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. *Frontiers in Neuroendocrinology* **32** 265–286. (doi:10.1016/j.yfrne.2010.12.001)

Received in final form 4 April 2013
Accepted 16 April 2013
Accepted Preprint published online 16 April 2013

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-12-0218

Published by Bioscientifica Ltd.