LOW BIRTH WEIGHT ASSOCIATES WITH HIPPOCAMPAL GENE EXPRESSION

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Abstract—Birth weight predicts the lifetime risk for psychopathology suggesting that the quality of fetal development influences the predisposition for mental disorders. The connectivity and synaptic network of the hippocampus are implicated in depression, schizophrenia and anxiety. We thus examined the underlying molecular adaptations in the hippocampus as a function of the fetal conditions associated with low birth weight. We used tissues from the non-human primate, *Macaca fascicularis*, to identify changes in hippocampal gene expression early in postnatal development associated with naturally occurring low compared with normal birth weight. Microarrays were used to analyze gene expression and DNA methylation in the hippocampus of five low- and five normal-birth weight neonates. Real-time PCR was employed to validate differentially expressed genes. Birth weight associated with altered global transcription in the hippocampus. Hierarchical clustering of gene expression profiles from 24,154 probe sets grouped all samples except one by their birth weight status. Differentially expressed genes were enriched in biological processes associated with neuronal projection, positive regulation of transcription and apoptosis. About 4% of the genes with differential expression co-varied with DNA methylation levels. The data suggest that low birth weight is closely associated with hippocampal gene expression with a small epigenetic underpinning by DNA methylation in neonates. The data also provide a potential molecular basis for the developmental origin of an enhanced risk for mental disorders. © 2016 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: DAVID, Database for Annotation, Visualization, and Integrated Discovery; LBW, low birth weight; NBW, normal birth weight; qPCR, quantitative real-time PCR.

Key words: low birth weight, hippocampus, DNA methylation, mental disorders, microarray.

INTRODUCTION

Fetal growth and development, reflected in birth weight, are influenced by the quality of the prevailing environment and predictive of later metabolic health (Barker et al., 1993a,b; Rich-Edwards et al., 1997). Low birth weight also associates with the risk for multiple brain-based disorders such as attention-deficit hyperactivity disorder (Bhutta et al., 2002), depression (Thompson et al., 2001; Alati et al., 2007), and schizophrenia (Abel et al., 2010) as well as with endophenotypes for mental disorders such as the thickness of regional cortical surface areas, total brain and caudate volumes and cognitive abilities (Raznahan et al., 2012; Walhovd et al., 2012). These effects are present across the birth weight spectrum and linked to tissue- and gene-specific epigenetic modifications (Meaney et al., 2007).

Alterations in DNA methylation have been associated with metabolic diseases like type 2 diabetes (Park et al., 2008) as well as mental disorders like schizophrenia and bipolar disorder (Dempster et al., 2011; Xiao et al., 2014). Importantly, DNA methylation can be influenced by internal and external environmental cues such as diet, stress and glucocorticoid secretion, in particular during critical periods of prenatal and early postnatal development (Meaney et al., 2007). Modifications of DNA methylation can be stable and may relate to life-long phenotypic consequences, potentially including increased risk for the development of disease (Gluckman et al., 2009). Whereas changes in gene expression and DNA methylation have been detected in postmortem brain samples of subjects suffering from psychiatric disorders (Grayson and Guidotti, 2013) as well as in response to clinically relevant early environments (McGowan et al., 2009; Provencal et al., 2012), little is known about the adaptations early in development that occur in clinically relevant brain regions in response to the conditions that associate with low birth weight. Such changes would potentially set up a mechanism for a predisposition for an increased risk of disease development established during fetal life.

The hippocampus is implicated in anxiety, depression, and schizophrenia (Small et al., 2011; Femenia et al., 2012), all of which associate with birth weight. Low birth weight within the normal range associates with decreased hippocampal volume (Buss et al., 2007) and impaired

performance on hippocampal-dependent tasks (Strauss, 2000; Kirkegaard et al., 2006; Gieling et al., 2012). Studies with rodent or non-human primate models show that prenatal exposure to elevated glucocorticoid levels impairs growth, resulting in a lower birth weight, and constrains hippocampal development (Uno et al., 1989, 1994; Coe et al., 2003; Lister et al., 2005).

We used the non-human primate, *Macaca fascicularis* to identify changes in hippocampal gene expression and DNA methylation early in development associated with naturally-occurring instances of low birth weight (LBW) as compared to normal birth weight (NBW). Our data suggest that impaired fetal growth associates with altered global hippocampal transcription which is not broadly accompanied with DNA methylation changes.

EXPERIMENTAL PROCEDURES

### Animals and collection of brain tissue

All animals were healthy. They were bred and sacrificed at the Nafovanny facility (Vietnam), which is a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Dams and offspring were exposed to the local climate. There were no differences in the diet between dams. All neonates in this study were female and from unique matings. They were sedated between 09:20 and 11:15 am with an intramuscular injection of ketamine-HCl (15 mg/kg), and exsanguinated under anesthesia. Whole brains were collected, flash frozen and stored at $-80\ ^{\circ}$C.

Tissue processing

Coronal sections from the right hippocampus were prepared in a series of two sections of 300 μm and 20 μm respectively by cryosectioning and thaw-mounted on polylysine-coated slides. Slides were stored at minus 80 °C until further processing for Nissl staining and nucleic acid extraction. Nissl staining facilitated the identification of the region of interest. 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 and Cowan (1984). The lateral geniculate nucleus was used as a landmark (Fig. 1). A standard protocol was used for Nissl staining of 20 μm brain sections. Pictures were taken with an Axio Observer Z1 microscope (Zeiss, Germany) and stitched with TissueFAXS suite version 3 software (TissueGnostics, Austria).

RNA and DNA extraction

The area used for nucleic acid extraction from the hippocampus is indicated in Fig. 1. Total RNA and DNA was isolated from a single 300 μm section using the All Prep DNA/RNA Micro Kit (Qiagen, Singapore) following the manufacturer's protocol, including DNase digestion for RNA samples. The nucleic acid samples were subjected to spectrophotometric measurement and the quality of RNA (RIN values) determined using an Agilent
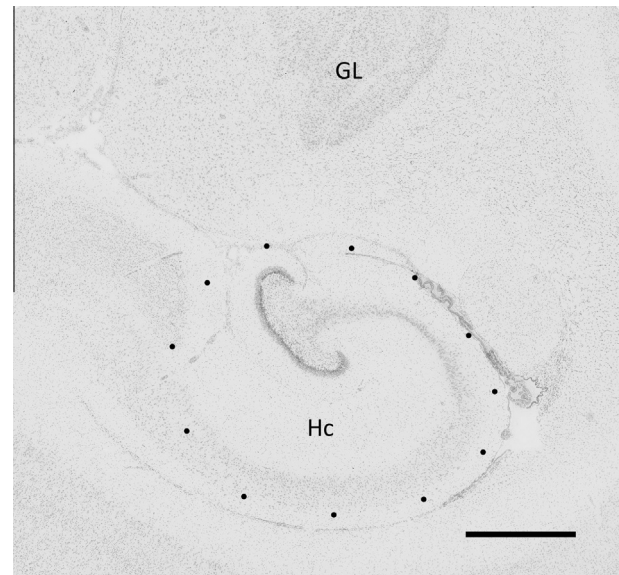
Fig. 1. Representative Nissl staining of the hippocampus from a female Cynomolgus macaque neonate at the age of 6.6 days. Black dots demarcate the region analyzed in our study; the scale bar represents 1 mm. GL, dorsal lateral geniculate nucleus; Hc, hippocampus.

Bioanalyzer. All RNA samples had an RIN value between 7.7 and 9.1.

Expression microarray

Five LBW and five NBW samples (plus a technical and a biological replicate) were hybridized to Affymetrix Rhesus Genome Arrays (ORIGIN LABS, Singapore). The RNA samples were processed with the Affymetrix 3′ IVT Express Target Amplification, Labeling and Control Kit according to the manufacturer's protocol. Briefly, 60 ng of total RNA was reverse transcribed to produce cDNA, which was subsequently used as a template to create biotin-labeled aRNA (amplified RNA). Four unlabeled, polyadenylated RNA spikes in different concentrations were included to assess the target preparation steps. The aRNA was then fragmented and hybridized to Affymetrix Rhesus Genome Arrays for 16 h at 45 °C with rotation at 60 rpm. Four pre-labeled bacterial hybridization controls were included as well. Arrays were washed and stained with the Affymetrix GeneChip Hybridization, Wash and Stain Kit and scanned using an Affymetrix 3000 7G scanner. All controls were identified on all arrays and showed signal intensities corresponding to their initial relative abundance (data not shown).

Expression microarray data analysis

Raw probe set intensities were normalized using the GCRMA (GC content – Robust Multi-Array Average) algorithm (Wu et al., 2004) in the Affymetrix R package and were log2-transformed. After filtering out probesets for which at least one sample was non-expressing (expression below 3.5), 24,154 probesets remained.