Effects of the cumulative exposure to Lypopolysaccharide and Hyperoxia on the developing rat brain assessed by high-field diffusion tensor imaging

Y. van de Looij[1,2], M. Sifringer[3], F. Brehmer[4], B. Gerstner[5], P. S. Hüppi[1], R. Gruetter[2,6], U. Felderhoff-Müser[7], and S. V. Sizonenko[1]

[1]Division of Child Growth & Development, Department of Pediatrics, University of Geneva, Geneva, Switzerland, [2]Laboratory for Functional and Metabolic Imaging, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [3]Department of Anesthesiology, Charité-Universitätsmedizin Berlin, Berlin, Germany, [4]Department of Neonatology, Charité-Universitätsmedizin Berlin, Berlin, Germany, [5]Department of Pediatric Cardiology, University Hospital Giessen, Giessen, Germany, [6]Department of Radiology, University of Geneva and Lausanne, Geneva and Lausanne, Switzerland, [7]Department of Pediatrics, University Hospital Essen, Essen, Germany

Introduction:

In premature infants, periventricular leukomalacia (PVL) is a common type of injury of the cerebral white matter resulting in a chronic disturbance of myelination. Two major causes are generally considered to be responsible for perinatal white matter injury: cerebral ischemia/reperfusion in the premature infant and bacterial infection in the mother and/or fetus [1]. Therefore, animal models of PVL can be achieved by hypoxia-ischemia as well as bacteria-derived lipopolysaccharide (LPS) exposure. Additional oxygen is widely used in resuscitation and in treatment of neonatal lung disease. Furthermore, premature infants are subjected much earlier to relative hyperoxia, because of a dramatic rise of oxygen tissue tension compared with intrauterine conditions. There is increasing evidence that hyperoxia may negatively influence brain development and maturation [2]. Yet, the association of a cumulative exposure to LPS and hyperoxia has never been evaluated and is the aim of the current study. Diffusion tensor imaging (DTI) derived parameters as directional diffusivities ($D_{//}$ and $D_{\perp}$) and Fractional Anisotropy (FA) are commonly used to probe brain microstructure. The goal of this study was to characterize changes in the pup rat brain following LPS and/or hyperoxia exposure by DTI derived parameters.

Materials and Methods:

3-days after birth (P3), rats were treated by intraperitoneal injection of LPS (0.25mg/kg) or vehicle solution (0.25mg/kg). At P6, rats were subjected to hyperoxia (80% O_2 for 24h) or normoxia (21% O_2 for 24h). At P21, rats were sacrificed and brains were formalin-fixed for subsequent *ex-vivo* MRI and histology resulting in 4 groups: LPS and Hyperoxia (LH), LPS and Normoxia (LN), Vehicle and Hyperoxia (VH) and Vehicle and Normoxia (VN = Sham). All experiments were performed on an actively-shielded horizontal 9.4T/31cm magnet (Varian/Magnex) equipped with 12-cm gradient coils (400mT/m, 120µs) with a transmit-receive 25-mm birdcage RF coil. After manual adjustment of the first and second order shims (water linewidth ~ 20 to 40 Hz) a Spin-Echo sequence with addition of the Stejskal-Tanner diffusion gradients was used. Diffusion gradients were applied along twelve spatial directions: dual gradient diffusion gradient sampling scheme [3] as well as the six opposite directions to cancel *b*-value cross terms [4]. Intensity, duration and diffusion time were set to 22 G/cm, 3 ms and 20 ms respectively, given a *b*-value of 1185 s.mm^{-2}. A field of view of 16 × 16 mm^2 was sampled on a 128 × 64 cartesian grid. Multi-slice DT images were acquired (12 slices of 0.5 mm thickness) in the axial plane with 10 averages and TE/TR = 30/2000 ms. Using in house Matlab script (Mathworks, Natick, MA), diffusivity values ($D_{//}$ and $D_{\perp}$) as well as FA was derived from the tensor. The program allows manual delineation of region of interest (ROI) on the FA maps. Four different regions of the brain were analyzed: the corpus callosum (CC), the external capsule (EC), the superficial layer of sensori-motor cortex (SCx) and the basal ganglia (BG) at six different levels of the brain (i.e. six image planes). Significant differences of diffusivity and FA values between the groups were assessed by a Mann-Whitney test.

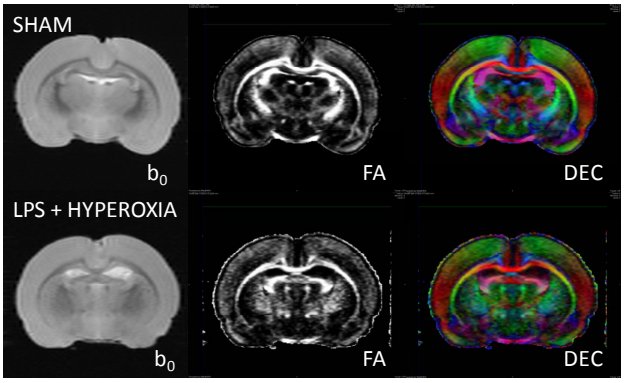

Fig. 1: T_2W images (b_0), fractional anisotropy (FA) and direction encoded color maps (DEC) of a typical VN (up) and LH (down) rat brain.

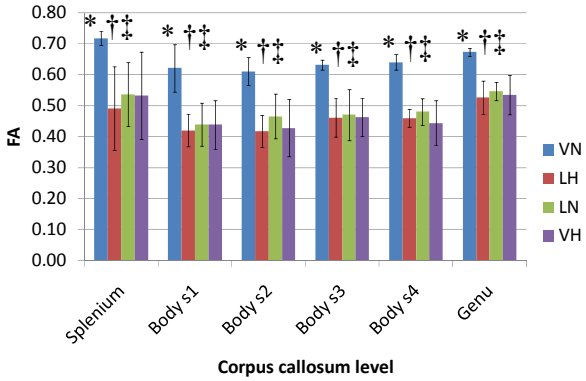

Fig. 2: FA ± SD at different levels of the CC: Splenium, Body slices 1 to 4 and Genu for the 4 groups: VN, LH, LN and VH. FA values were significantly decreased in the CC of LH, LN, and VH compared to VN (* †‡: $p<0.05$ for VN vs. LH, LN and VH respectively).

Results and Discussion:

Typical DT images are presented in Fig. 1. Significant decrease of FA (Fig. 2) was observed in the corpus callosum of the injured groups (LH, LN,VH) compared with the sham group (VN) due to a significant increase of $D_{\perp}$ (i.e. myelination defect). There were no significant differences between the injured groups providing evidence of similar damage in corpus callosum due to hyperoxia alone or LPS with or without hyperoxia. For only one of the six image planes, FA values were found significantly lower in the cortex of the LH group compared with Sham meaning cortical damage only for the cumulative LPS and Hyperoxia group. To conclude, our study confirmed white matter damages following LPS injection alone, hyperoxia alone as well as cumulative LPS and hyperoxia exposure. Hyperoxia in addition to LPS induced PVL did not seem to enhance white matter damages but hyperoxia alone also induces white matter damages.

References: [1] Volpe JJ. Lancet Neurol 2009; [2] Felderhoff-Mueser U. NeuroBiol of Dis 2004; [3] Basser PJ. MRM 1998; [4] Neeman M. MRM 1991.

Acknowledgements: Supported by NEOBRAIN Consortium (EC-6thFP) European Commission, the Fond National Suisse (N° 31003A-112233-Switzerland), the Centre d'Imagerie Biomédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV, EPFL, the Leenards and Jeantet Foundations.