Investigation of metabolite changes in neonatal rat brain after cerebral hypoxia-ischemia using 1H-spectroscopy at 9.4T

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Introduction The pathogenesis of brain lesions associated with prematurity is not yet well understood. Brain injury results not only in loss of cellular elements but also triggers normal brain development alterations. The aim of this study was to investigate acute and long term brain alterations after neonatal hypoxic-ischemic (HI) injury in the 4-day old rat pup using MR imaging and spectroscopic techniques.

Materials and Methods 3-day old Sprague-Dawley pups underwent moderate hypoxic-ischemic (HI) injury under isoflurane anesthesia as follows: a ligature of the right carotid artery was performed and rat pups were kept under hypoxia for 30 minutes at 6% O_2. 24-hours after HI, each rat pup was placed supine within an adapted rat holder to perform the MR scans. Throughout the MR experiment, they were continuously anesthetised under a flow of 1.5-2% isoflurane in oxygen. The rat body temperature was controlled through a rectal probe and was maintained at 37^0C using a thermoregulated water circulation placed under the rat bed, respiration and heart rate were monitored during the experiment. Eight (8) rat pups were studied 24 hours after HI. 4 of them were scanned again 21 days after HI. For comparison purposes, 4 healthy 7-day old rats were also investigated as well as 4 healthy 21-day old rat pups under the same conditions.

All experiments were performed on a actively shielded 9.4T/31cm magnet (Magnex Scientific, Abington, UK) interfaced to a Varian INOVA console (Varian, Palo Alto, CA) equipped with 12-cm gradient coils(400mT/m, 120us). A quadrature transmit/receive surface RF coil consisting of two geometrically decoupled 17 mm single-turn coils was used. First and second order shims were adjusted for each volume of interest (VOI) of size 2x3x3 mm^3 using FASTMAP(1). The water signal was efficiently suppressed using VAPOR (2) and spectra acquisitions both within the cortical lesion and the contralateral cortical area were performed using STEAM (TE/TM/TR= 2.8,20, 4000ms) with outer volume suppression (OVS). 20 to 30 series of FIDs were acquired, individually corrected for frequency drifts, summed and corrected for residual eddy current effects using the reference water signal. The position of the VOI was based on Fast Spin Echo (FSE) MR images (TR/TE= 4000/96ms; 16 echoes; ΔTE=12ms; slice thickness= 1 mm; 8averages; FOV= 25x25mm; matrix=256x256).Proton spectra were analyzed with LCMODEL(3) using the unsupressed water signal corrected for age-dependent changes in brain water content (4) as an internal reference. Metabolites were quantified in both the cortical lesion and the cortical contralateral part, 24 hours after HI induction (4 day old pup (P4)). They were also quantified in the healthy cortex in 7 day old (P7) and 21-day old pups (P21). A paired t-test was used to compare metabolite concentrations measured between the lesion side and the contralateral side.

Results 4 T_2-weighted FSE images of a 4-day old rat pup brain show (Figure1) the edematous HI lesion induced the day before (Arrows). 21 days after HI induction. the rat brain evolved to a highly distorted and asymmetric structure where the striatum partially replaced the ipsilateral cortical tissue (figure2). An example of ^{1}H-NMR spectra of the cortex acquired at P4 is given (figure 3). A comparison of the neurochemical profile between the lesion side and the contralateral side at P4 is shown (figure 4). At P4, all the metabolite concentrations of the lesion side were lower than in the contralateral normal cortex although not statistically significant ($p>0.05$) except for myo-inositol ($p<0.05$). Interestingly, metabolite concentrations in the normal cortex of rat pups acquired at P4 and P7 were similar suggesting that most changes occur in rat brain on or after P10.

Discussion. The current study extends the characterization of the neurochemical profile in the developing rat brain to an earlier time point, namely P4. In addition the current study confirms previous measurements performed at P7 and P21 (Fig5) (4). The observation that 24h after HI injury, absolute metabolite concentrations were normal, in conjunction with the displacement of the ipsilateral cortex 2 weeks later implies that the biochemical processes indicating tissue destruction occur between P4 and P21 in our model.

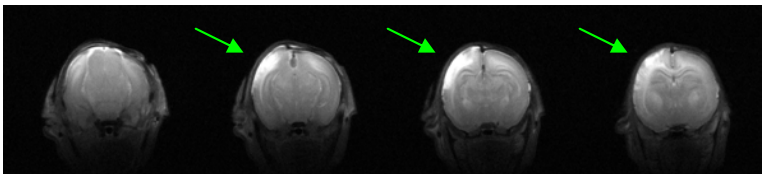

Fig1:4 FSE slices of a 4-day old rat pup, 24H after HI injury. Arrows indicate brain injury.

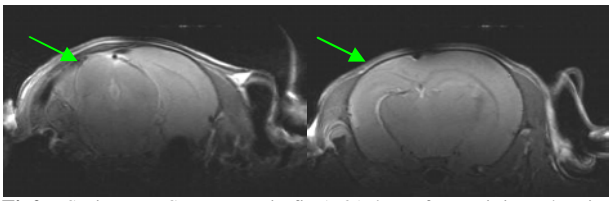

Fig2: FSE images. Same rat as in fig 1, 21 days after HI injury showing brain distortions

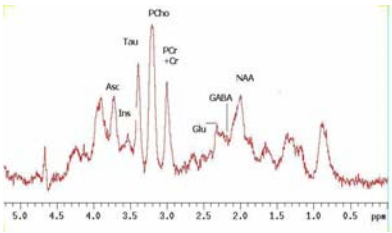

Fig 3: Typical 1H NMR spectrum of the cortex of a 4-day old rat pup.

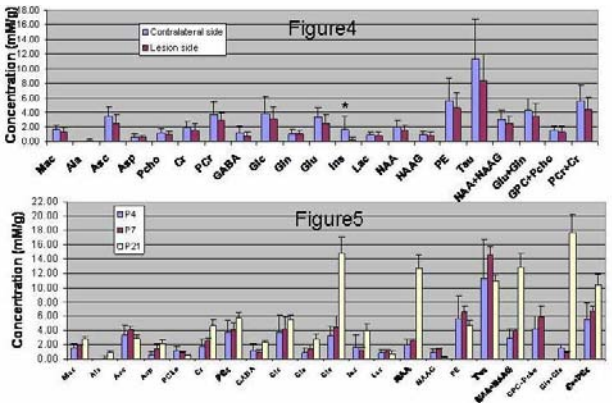

Fig 4: Comparison between the quantified metabolite absolute concentrations measured in the HI lesion and the contralateral area of the 4-day old rat pup cortex (n=8).**Fig 5:** Metabolite concentration changes at P4, P7 and P21 during brain development in normal cortex.

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