New Insights into Mouse Brain Maturation as Assessed by ^{1}H MRS at 7 Tesla

B. Schmitt[1], C. Amara[2], I. von Both[3], P. Bachert[1], and A. Schulze[3,4]

[1]Department of Medical Physics in Radiology, German Cancer Research Center (DKFZ), Heidelberg, Germany, [2]Faculty of Physical Education and Health, University of Toronto, Toronto, Canada, [3]Research Institute, Hospital for Sick Children, Toronto, Canada, [4]Department of Pediatrics, Hospital for Sick Children, Toronto, Canada

Introduction:

Metabolic diseases are genetic conditions that often manifest in infancy or childhood. Diagnosis and treatment have to be accomplished early after birth to prevent patients from serious and irreversible impairment. In order to optimize treatment, metabolic pathways involved in the disease as well as the effect of different treatment strategies have to be well understood. Localized proton magnetic resonance spectroscopy (^{1}H MRS) has proven to be a useful tool for the non-invasive assessment of brain metabolism. Single-voxel MRS has previously been applied in animals and in humans to examine regional and age-dependent changes in brain metabolites during development [1-3].

We performed an animal MRS study to obtain information on metabolite pattern in mouse brain maturation and for biochemical phenotyping the brain of a commonly used mouse strain.

Materials & Methods:

Animals and Monitoring: The study was performed in accordance with Canadian animal guidelines after approval by the responsible Animal Care Committee. C57Bl/6 mice newly born in the housing facility were kept with the mother throughout the study time until postnatal day 26 (p26). Four animals were examined at four different time points to monitor the course of brain metabolite levels. Mice were positioned (prone) on a murine slider bed (Bruker Biospin, Ettlingen Germany) with respiratory monitoring (SA Instruments, Stony Brook, NY, USA). Anaesthesia was introduced and maintained by inhalation of a mixture of oxygen and isoflurane while keeping the animal breathing spontaneously during the measurement. The animal's body temperature was maintained by water heating of the animal bed to 39.5 °C.

Proton MRI and MRS: All experiments were performed at 7 T on a Bruker BioSpec 70/30 USR MR system (Bruker Biospin, Ettlingen, Germany). A linear 7.2 mm inner-diameter volume resonator was used for RF excitation, while signal was acquired with a mouse brain surface coil (Bruker). A set of transversal RARE images was acquired prior to voxel positioning for MRS. The voxel (2.5x1.4x2 mm^3) was placed in the thalamus region while trying to avoid contributions from tissue-tissue borders or ventricular spaces. Localized shimming usually led to linewidths of 10-13 Hz (FWHM). A point-resolved spectroscopy (PRESS) sequence with VAPOR water signal suppression (TR/TE/NEX=2500 ms/20 ms/256) was used for the acquisition of spectral data. Each spectrum was analysed by LCModel [4] using the unsuppressed water signal as a reference for metabolite quantification. A mean brain water content of 43.7 mol/l [5] was assumed for an adult mouse to calculate water content in younger animals according to [1]. Statistical differences in metabolite concentrations in subsequent time-points were analysed by a paired *t*-test.

Results and Discussion:

The survival rate of the animals after anaesthesia was 100 % and their development did not exhibit any apparent abnormality.

Resonances of tNAA (NAA+NAAG), tCho (GPC+PCh), tCr (Cr+PCr), Tau, Glx (Glu+Gln), and Ins could be detected in each spectrum with estimated standard deviation < 6 % (except for Ins) as a result of good spectral quality (Fig. 1). SNR of spectra was in most cases larger than 10. Metabolite levels of all animals did not show large individual differences except for Glx and *myo*-Ins. In the case of Glx this is attributed to difficulties to separate the signal from overlapping resonances. Especially because the Glx time courses in individual animals did not exhibit any constant trend, we conclude that the development of Glx distribution is highly variable. In this study, Ins had the lowest sensitivity with higher SD values (10-15 %) compared to other metabolites.

Even though time courses of metabolite concentrations have previously been reported for the developing mouse brain until p21 [1,3], further progression as well as the transition point to a fully mature brain have not yet been assessed to our knowledge. While tCho, tNAA, Tau and tCr concentrations do not change significantly after p21, a further increase was observed for Ins (Fig. 2). The steady increase in Ins until p26 and the trend in persistent decrease of Tau suggest that mouse brain development is continuing beyond p21. We assume that this development is rather functional than structural as the main function of Ins is in osmoregulation [6] and that of Tau in the regulation and protection of neuronal connections [7].

Conclusion:

The study gains knowledge about physiological changes in the metabolic pattern of the maturing mouse brain which is important for the investigation of treatment strategies in order to differentiate between effects of treatment and physiologic processes. Because of morphologic and metabolic differences in the phenotype of commonly used mouse strains [5,8] the choice of a suited animal model is crucial to study a disease.

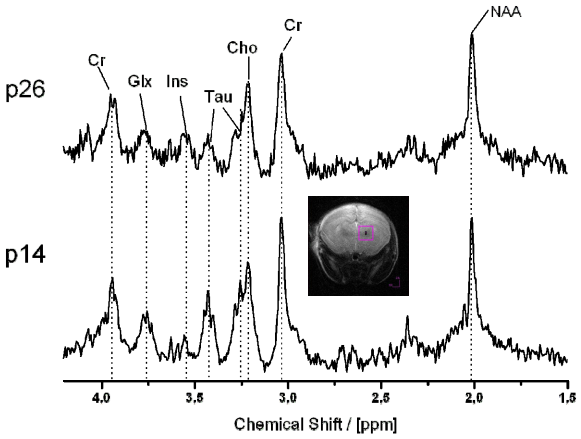

Fig. 1: *In vivo* ^{1}H MR Spectra from mouse brain at p14 and p26, voxel (7 mm^3) in the thalamus.

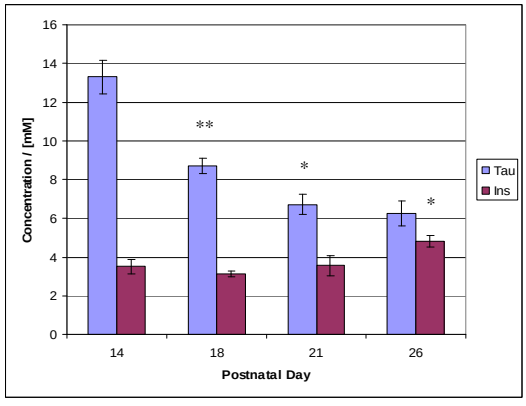

Fig. 2: Time course of the concentrations of Ins and Tau in the thalamus of mice from p14 to p26. Values are mean values (n=4) with SEM as error bars. **P*<0.05, ***P*<0,01 paired *t*-test between subsequent time points.

References:

[1] Larvaron P et al. NMR Biomed 2006:19
[2] Pouwels PJ et al. Ped Res 1999;46(4)
[3] Weiss K et al. MAGMA 2008
[4] Provencher SW. Mag Res Med 1993;30
[5] Schwarcz A et al. Mag Res Med 2003;49
[6] Thurston JH et al. Ped Res 1989;26
[7] Janáky R et al. Amino Acids 2008;34
[8] Penet M-F et al. Behav Genet 2006;36