Feasibility of high throughput – high performance ^{1}H MRS in baby-mice

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PURPOSE

The neurochemical profile of a mouse brain is changing extremely fast during the brain development period, especially between postnatal days 10 (P10) and P20. The difference in postnatal age by a single day affects the final metabolite quantification. Ideally, all mice in the studied litter should undergo the MRS scan the same day to avoid any bias caused by the age related neurochemical changes. Therefore, developmental studies with mice have high demands on used MRI/ MRS protocol in order to decrease the time requirements to scan one animal and to increase the throughput of the experiment. The purpose of this study was to optimize the whole MRS experiment designed for neonatal mice in order to maximize the throughput without compromising the MRS data quality. The outcome of this optimization is demonstrated by using a mouse model of neonatal anemia.

METHODS

Measurements were performed on a 9.4T magnet (Agilent/Vaian) equipped with a powerful gradient/shim coil (Resonance Research). A quadrature double-loop surface RF coil (loop size 8 mm) was used for the transmission and reception. The multislice FSE imaging was used for the VOI selection. FASTMAP shimming and ultra-short TE STEAM (TE = 2 ms) localization sequence combined with VAPOR water suppression were used for ^{1}H MRS[1,2]. Metabolites were quantified using LCModel with the spectrum of fast relaxing macromolecules included in the basis set. During the MRI/MRS experiment spontaneously breathing animals were anesthetized with 1.0–1.5% isoflurane. The respiration was monitored using SAM PC (Small Animal Instruments). The anemia was induced by a periodic blood draws starting on P3 which resulted in a drop of hematocrit to 21 ± 1 (control group 32 ± 1). The body weights on the day of MRS scan were 6.6 ± 0.4 g for control group (N = 6) and 6.4 ± 0.4 g for anemic group (N = 6).

RESULTS

The experimental protocol was optimized to reduce the time requirement for scanning one baby-mouse below 60 min, including anesthetizing mouse, fixing in a holder that has embedded respiration monitor, tuning the RF coil and running the whole MRI/MRS experiment. An optimized automatic B_0 shimming and MRS parameter adjustment (only the transmit power in the VOI) reduced time requirements for adjustments below 3 min. Despite very high B_0 inhomogeneity in the small mouse head, FASTMAP shimming combined with a strong 2nd-order shim system guaranteed excellent B_0 homogeneity resulting in unsuppressed water signal linewidth of 8.8 ± 0.3 Hz. The quality of ^{1}H MRS data is demonstrated in Fig. 1, where all acquired spectra from 6 anemic and from 6 control mice are overlaid. The quality of shimming is well documented by resolved resonances of creatine and phospohocreatine at 3.9 ppm. This data quality enabled a reliable quantification of 16 brain metabolites (Fig. 2). Small, but significant differences between anemic and control mice were found for lactate and phosphoethanolamine.

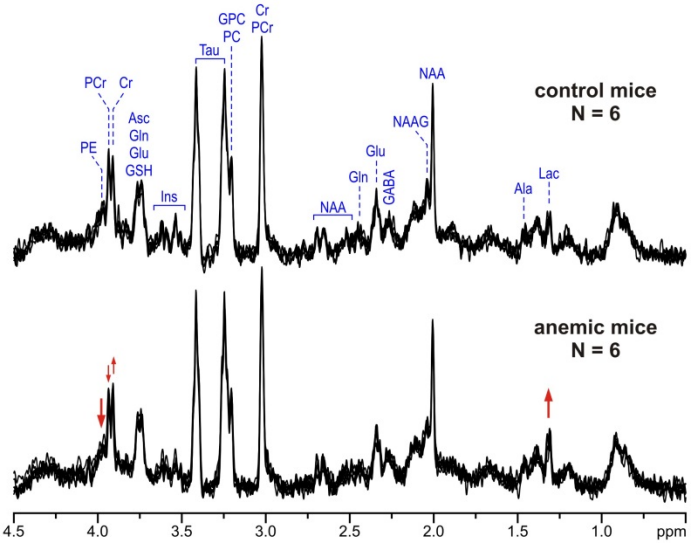

Figure 1 Overlay of all ^{1}H MR spectra from the hippocampus of anemic and control P14 mice measured in one study session.

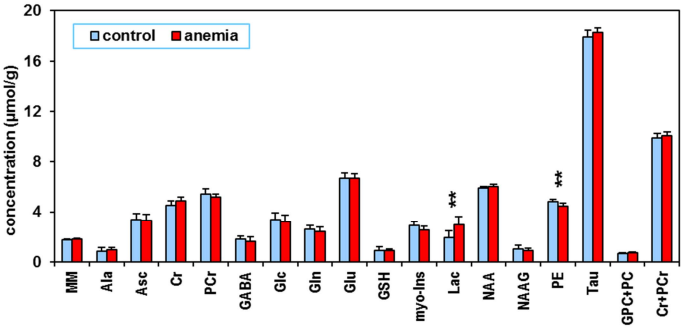

Figure 2 Hippocampal neurochemical profile of anemic mice (N = 6) and their littermate controls (N = 6) on postnatal day 14. Error bars indicate SD, ** p < 0.02.

DISCUSSION

The results of this study clearly demonstrate that high throughput/high quality ^{1}H MRS is feasible in neonatal mice. An optimized mouse holder decreased the time requirement for animal handling and guaranteed the correct position of the mouse head in the magnet. The small RF coil substantially increased the sensitivity and improved the SNR of spectra despite very small VOI = 3 μL. Automatic adjustments (shimming, power calibration, water suppression) required just a few minutes which also contributed to shortening the scanning time. All these procedures for speeding up the experiment did not compromise the data quality (Fig. 1, 2). Such a protocol enabled to scan 12 mice in a single session. Observed changes in Lac and PE are consistent with changed redox potential and altered myelination resulting from iron deficiency[3].

CONCLUSION

High throughput/high performance ^{1}H MRS is feasible in neonatal mice which enable to acquire data from the whole litter at the same age.

References: 1. Gruetter and Tkac, *Magn Reson Med* 2000: 43, 319; **2.** Tkac et al., *Magn Reson Med* 1999: 41, 649; **3.** Rao et al. *Pediatr Res* 2013: 73, 31.

Supported by: NIH grants P41 EB015894, P30 NS5076408, R01 HD029421 and the WM KECK Foundation