A metabolic study of normal mouse brain maturation using hyperpolarized 13C

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Introduction: Dynamic nuclear polarization (DNP) has been proven to increase ^{13}C NMR signal for more than 10,000 fold, allowing investigations of ^{13}C metabolic exchanges in vivo[1,2], in contrast to T1- and T2-weighted MRIs that can assess morphological changes. Using DNP and T2-weighted MRI, our study investigated the changes in pyruvate to lactate conversion and voxel-based volume changes in relation to maturation in mice.

Methods: 8 normal mice were scanned starting on postnatal day 18 and repeated every 10 days. Some time points were delayed due to technical issues. Mice were anesthetized with 1.5% isoflurane and 1 L/min oxygen during scans. All experiments were conducted on a vertical 14.1T (Agilent) 600WB NMR spectrometer with 55mm 1000mT/m gradients and a 40mm diameter ^{1}H and ^{13}C dual-tuned coil. C1 labeled ^{13}C pyruvate was polarized using an Oxford Hypersense^{TM} DNP instrument and 150μL of the dissolution mixture containing 160mM pyruvate was injected into the tail vein through a catheter over a span of 12 seconds. **Data acquisition:** Data were acquired on a 24 mm × 24 mm × 5 mm slab centered on the brain, with 2D chemical-shift imaging acquired using center-out 8x8 phase encoding with 128 spectral points. The acquisition was started simultaneously with the pyruvate injection and repeated every 4s (3s TR with 1s delay between each repetition) for a total of 60s with constant flip angle of 10°. A T2-weighted image was acquired using fast spin echo with TR/TE=1.3s/12ms and resolution of 0.12mmx0.12mmx1mm. **Data processing:** (1) DNP data: A 5Hz Lorentzian apodization was applied to each free-induction decay before Fourier transforming the data. Maximum intensity of pyruvate and lactate was recorded at each time point for each pixel. We choose the six pixels matched by the location from the T2 anatomical image for calculation of pyruvate and lactate from the average signal intensity of the six pixels. To normalize polarization levels and compare lactate signals with age, lactate signal was divided by the total carbon signal in the slab at each time point. The average of the 8 subjects were taken at each time point to observe a general trend of the lactate level change with age. We also recorded the peak height of normalized lactate in each scan for every mouse. (2) T2-weighted MRI: For each individual, we linearly co-registered brains of two consecutive scans (i.e., 1st to 2nd, 2nd to 3rd, and so on). We then computed brain volume changes between the two scans using the multiplication of the three scaling parameters (in x, y, z-axes) in the resultant transformation. **Statistical analysis:** To examine the association between age and the lactate level or brain volume, we fit a mixed effect linear model on the normalized lactate peak and the volume scaling using SurfStat[3]. This model permitted multiple measurements per subject while controlling for between-subject variation, thus increasing statistical power.

Results: As seen in Figure 1, the normalized lactate signal decreased with increase in age. At young age (P18-P22), a significant higher lactate level can be observed. The normalized lactate peak level was attenuated in relation to maturation (t=-3.84; p=0.001; Figure 2). Linear regression on volume scaling showed no relationship between volume and age (t=0.82; p>0.422).

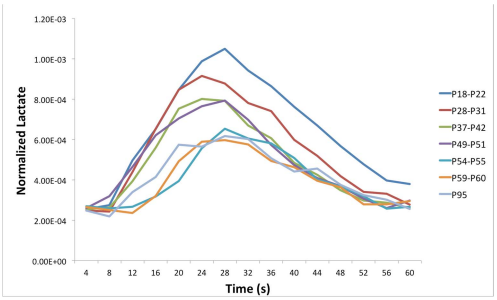

Fig 1. Normalized lactate intensity averaged among 8 mice at each age range.

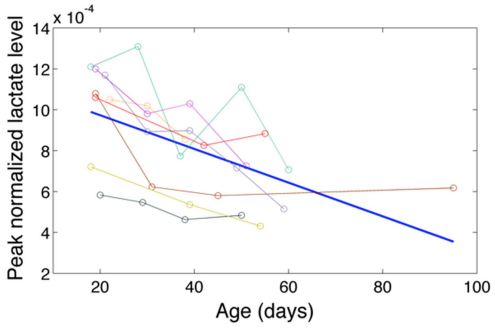

Fig 2. Normalized lactate intensity of each mouse with linear regression.

Discussion: Higher pyruvate to lactate conversion in earlier maturation stage that was found in DNP analysis may explain greater utilization of lactate as energy[4]. Consistent with previous ex vivo MRI study[5], our volumetric analysis showed no global brain volume change after P18 in mice, suggesting slow structural maturation after early postnatal period, while metabolic maturation is still ongoing. The proposed analytic framework and the observed maturation pattern in healthy mice can be potentially useful to assess brain maturation in cases with early injury model such as derived from hypoxia.

Reference: 1. Ardenkjaer-Larsen J, et al. (2003) *PNAS* 100, 10158-10163. 2. Golman K, et al. (2006) *PNAS* 103, 11270-11275. 3. Worsley KJ, et al. (2009) *NeuroImage 47*, S102. 4. Pellerin L, et al. (1998) *PNAS* 95, 3990-3995. 5. Chuang N, et al. (2011) *NeuroImage* 54, 80-89.