^{1}H-MRS profiling of the developing rat brain

S. Liachenko[1], and J. Ramu[1]
[1]FDA / NCTR, Jefferson, AR, United States

Introduction

Neurotoxicity is an important outcome to consider when assessing new drug safety and environmental toxicity, especially in the pediatric population. Currently, developmental neurotoxicity is often assessed using traditional ex vivo methods such as histopathological or biochemical evaluations. However, with the rapid development of non-invasive in vivo imaging/spectroscopy techniques, there is an opportunity to optimize the efficiency and use of animals in toxicological studies using these methods longitudinally. In the current study, naïve rats were assessed using ^{1}H-MRS during early development beginning from 2 weeks of age to provide a baseline for developmental toxicology research.

Methods

Animal handling and MRI/MRS procedures were approved by our local IACUC. Twelve postnatal day (PND) 7 Sprague-Dawley rats from the local breeding colony were housed in plastic isolators with hardwood chip bedding. Animals received food and water ad lib and were maintained on a 12 hr/12 hr light/dark cycle, with testing during the light phase. MRS was conducted using a BioSpec 7T/300USR MRI system (Bruker BioSpin, Billerica, MA) with a 12 cm ID gradient insert (600 mT/m) and a 72 mm quadrature volume transmit and 4-channel phased array rat brain optimized receive-only surface RF coil (Bruker BioSpin, Billerica, MA) under general isoflurane anesthesia at a controlled rat core temperature (37.1 ± 0.6°C). The spectroscopic voxels were positioned at the left dorsal hippocampus (HC, 4×4×2 mm) and anterior cingulate cortex (CC, 2.5×2.5×2.5 mm) using a fast spin echo (RARE) brain image as a reference. The magnetic field homogeneity in these voxels was adjusted separately using FASTMAP to yield a FWHM of 10-12 Hz. Proton MRS was performed using PRESS localized sequence with VAPOR water suppression and outer volume saturation with the following parameters: TE = 8 ms, TR = 2.5 s, NS = 256. Resultant spectra were analyzed using LCModel (1) and separate water-unsuppressed spectra (NS = 8) were used for eddy currents correction and metabolite concentrations calibration. Each rat was scanned at least 10 times beginning at PND 14 to 19 with the intervening periods of at least 1 week between scans. The scans were also staggered to cover the time span evenly from PND 14 to the end of the study. The data were analyzed using two-way ANOVA with SNK post-hoc tests (SigmaStat, Point Richmond, CA).

Results and Discussion

LCModel output has shown a SNR of 6-13 for CC and 11-22 for HC. In particular, the %SD values for Glu in HC and CC were approximately 3% and 10%, respectively. The concentrations of creatine (Cre), N-acetylaspartate (NAA), glutamate (Glu), glutamine (Gln), GABA, myo-inositol (Ins) and choline (Cho) increased while the concentration of taurine (Tau) decreased with age, reaching plateaus at approximately PNDs 40-50 (Fig 1), which is in a very good agreement with ex vivo findings (2). The changes in Cre and Ins were more rapid in the HC, and the changes in Tau, Glu, and Gln were more rapid in CC. There were no regional differences in the concentration of Cho, NAA, and GABA. The data suggest that some brain neurochemistry becomes relatively stabilized by PND 40 in rats. Because Ins is predominantly linked to glial and Glu/Gln to neuronal activity, the difference in the dynamics of these metabolites likely reflects the heterogeneity of maturation process throughout the brain. These kinds of data may serve important roles in translational developmental neuroscience and as potential biomarkers for the developmental neurotoxicity studies in many drug safety evaluation programs. (Supported by NCTR/FDA, #P00731).

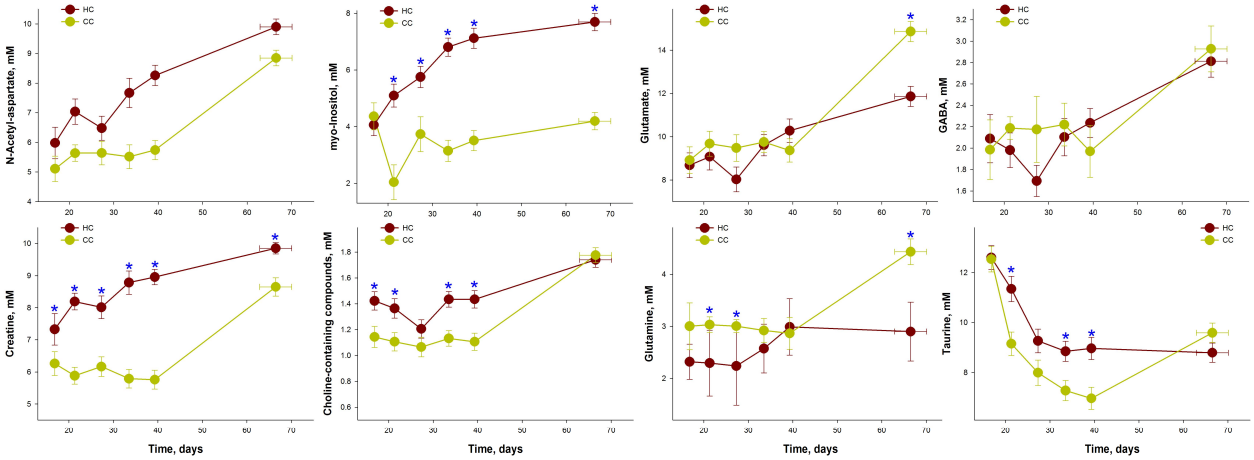

Figure 1. Time course of ^{1}H-MRS-detected metabolites in the developing rat dorsal hippocampus (HC) and anterior cingulate cortex (CC). Data were binned to discrete time points for clarity. Data are means ± SEMs. * = significant difference between brain regions ($P < 0.05$).

References

1. Provencher, S. W. (2001) NMR Biomed, 260-264.
2. Burri R., et al (1990) Neurochem Res, 1009-1016.