Developmental changes in neurochemical profiles of the mouse midbrain and hippocampus

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PURPOSE: Rodents' brains go through major developmental changes within the first four postnatal weeks. Changes in cellular composition and morphology, network architecture and metabolism are directly related to brain functionality. The timelines of these neurodevelopmental changes are, in general, regionally specific[1]. Developmental changes in the neurochemical profiles of selective brain regions were measured non-invasively by ^{1}H MRS in rats[2,3] and mice[4,5]. The purpose of this study was to investigate whether unique regional neurochemical changes occur during development and maturation of the midbrain and hippocampus - the brain regions that have not been characterized by ^{1}H MRS yet.

METHODS: Six C57BL/6 mice were scanned at P10 and P28 (postnatal day 10 and 28). All measurements were performed at 9.4T (Agilent/Varian) using FASTMAP shimming[6] and ultra-short TE STEAM (TE = 2 ms) localization sequence combined with VAPOR water suppression[7]. Metabolites were quantified using LCModel with the spectrum of fast relaxing macromolecules included in the basis set. The multi-slice FSE imaging (slice thickness = 0.8 mm) in coronal and sagittal orientations were used for a precise VOI selection.

RESULTS: The spectral quality consistently achieved in this study (Fig. 1) enabled reliable quantification of 15 - 17 brain metabolites and minimized the CV of their concentrations (Fig. 2). The difference in neurochemical profiles between midbrain and hippocampus were distinct at P10, which became even more obvious at P28. The characteristic features of P10 neurochemical profiles were high levels of phosphoethanolamine (PE) and taurine (Tau) and low levels of glutamate (Glu) and NAA. The signal of urea was clearly detectable at 5.8 ppm in P10 spectra (Fig. 1A) and its concentration was estimated to be 1.3 μmol/g. The key differences between these brain regions were high GABA and *myo*-inositol (Ins) and low Tau in the midbrain relative to hippocampus. The neurochemical profiles changed dramatically between P10 and P28. A highly significant increase in 7 metabolites (creatine, phosphorcreatine, glutamine, Ins, lactate, NAA) and decrease in 3 metabolites (N-acetylaspartylglutamate, PE, Tau) were observed in both brain regions between P10 and P28. In addition, decreased ascorbate and increased total cholines (GPC+PC) were measured in the midbrain at P28 relative to P10.

DISCUSSION: Observed developmental changes in metabolite concentrations are in very good agreement with the overall pattern of changes previously reported from rats[2,3] and mice[4,5], but the developmental changes in neurochemical profiles of the mouse midbrain and hippocampus were quantified for the first time. The high level of GABA detected in the midbrain is in agreement with in vitro biochemical data[8]. Particularly novel is the detection of urea in mouse brain at P10 (Fig. 1A). Urea concentration was probably underestimated due to water suppression and dynamic proton exchange. The brain metabolism does not include the urea cycle, but the urea presence in the brain is facilitated by the permeability of the blood-brain-barrier in early postnatal period. The higher level of Ins, lower level of Tau and smaller relative developmental increase in Gln and Glu in the midbrain relative to hippocampus indicate that the developmental timeline of this brain region is shifted to an earlier postnatal period relative to the hippocampus.

CONCLUSION: The neurochemical profiles midbrain and hippocampus will serve as a reference for further mouse models of neurodevelopment.

REFERENCES: **1.** Stiles et al., *Neuropsychol Rev* 2010:20,327; **2.** Tkac et al., *Magn Reson Med* 2003:50,24; **3.** Terpstra et al., *NMR Biomed* 2010:23,1038; **4.** Kulak et al., *J Neurochem* 2010:115,1466; **5.** Duarte et al., *Biol Psychiatry* 2012:71,1006; **6.** Gruetter et al., *Magn Reson Med* 2000:43,319; **7.** Tkac et al., *Magn Reson Med* 1999:41,649; **8.** Hedner et al., *J Neural Transmission* 1984:59,105.

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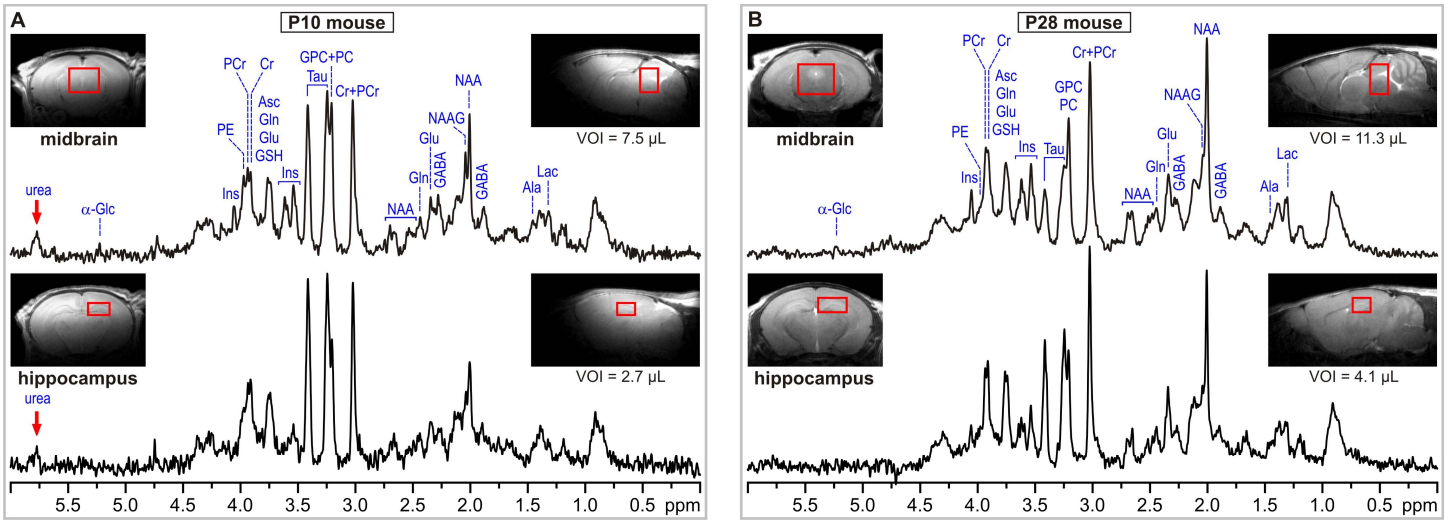

Figure 1 In vivo ^{1}H MR spectra acquired from the mouse midbrain and hippocampus at postnatal day 10 (A) and 28 (B). STEAM, TE = 2 ms, TR = 5 s.

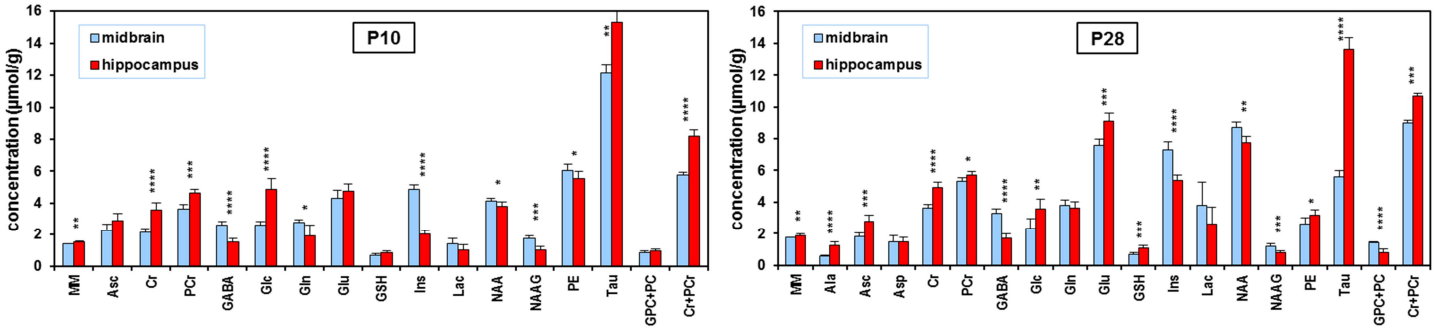

Figure 2 Neurochemical profiles of the midbrain and hippocampus at P10 and P28. Error bars: SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.