Neurochemical profile of hippocampal-specific iron transporter Slc11a2 (DMT-1) knock-out mice

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INTRODUCTION Iron deficiency (ID) is the most prevalent micronutrient deficiency in the world, affecting infants in both industrialized and developing countries. Infants are at greatest risk for ID during late gestation and early infancy due to severe maternal ID and pregnancies complicated by intrauterine growth restriction or diabetes mellitus [1,2]. Animal models of early ID show variations in the neurochemical profile and cognitive deficit [3,4]. However, these dietary models of ID may be confounded by anemia and affect multiple systems in the rat brain, which makes interpretation difficult. The aim of this study was to investigate consequences of hippocampal-specific ID in double knock-out mice using in vivo ^{1}H NMR spectroscopy at 9.4 T.

METHODS CamKIIa-cre mice were crossed with Slc11a2 flox/flox mice in order to achieve double-mutant mice with knock-out of the iron transporter Slc11a2 in forebrain neurons. Behavioral tests were performed using the Morris water maze. NMR measurements were performed using a Varian INOVA spectrometer interfaced to a 9.4 T magnet, equipped with powerful gradient/shim coils insert (Resonance Research Inc). First and second order shims were adjusted by FASTMAP [5]. Ultra-short echo-time STEAM (TE = 2 ms) combined with outer volume suppression and VAPOR water suppression was used for ^{1}H NMR spectroscopy [6]. Metabolite concentrations were quantified using LCModel with macro-molecule spectra included in the database and the unsuppressed water signal was used as an internal reference [7]. Spontaneously breathing mice (45-days-old, 14-month-old, n = 6 in both KO and WT group) were anesthetized by a gas mixture N_2O : O_2 = 1 : 1 with 1.5% isoflurane.

RESULTS AND DISCUSSION Iron content was selectively reduced in hippocampus of knock-out (KO) mice by 40% relative to wild type (WT) controls, without changing the hematocrit. Behavioral tests showed cognitive deficit of KO mice relative to WT. NMR spectra were measured from left hippocampus (Fig. 1) and 17 brain metabolites were reliably quantified from each spectrum. Of all metabolites quantified in hippocampus only Lac levels were significantly reduced in KO mice relative to WT controls (Fig. 2). Significantly reduced Lac was observed in both age groups. To observe the reduced Lac in ID KO mice was counterintuitive, but it can indicate a reduced glycolytic flux as a result of adaptation to the reduced capacity of TCA cycle and electron transport chain in mitochondria, due to the deficiency of iron inevitable for cytochrome enzymes. Lactate levels as an indirect measure of the oxidative metabolism flux under conditions of sufficient oxygen supply was recently suggested by Mangia [8]. Reduced energy production in hippocampus can explain observed deficit in cognition performance. In conclusion, developed hippocampal-specific ID mouse model has a potential to understand the impact of ID on the development and functionality of hippocampus.

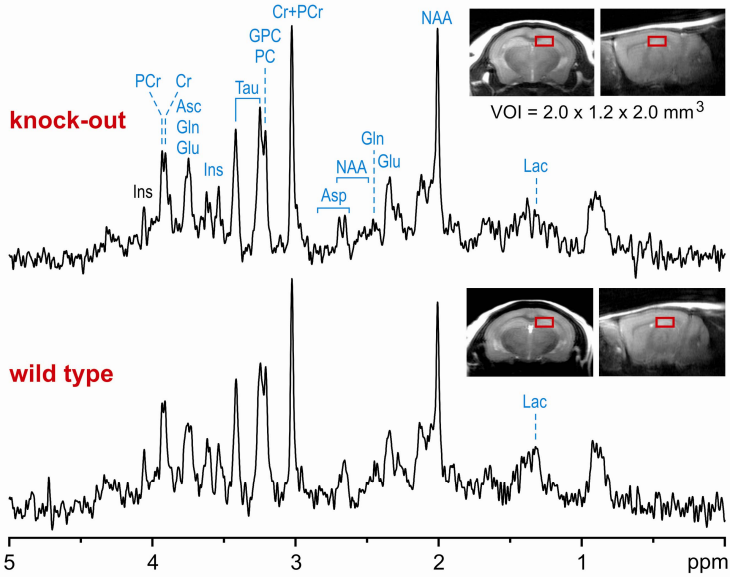

Fig. 1 In vivo ^{1}H NMR spectra measured from hippocampus of hippocampal-specific iron deficient KO mice and WT controls

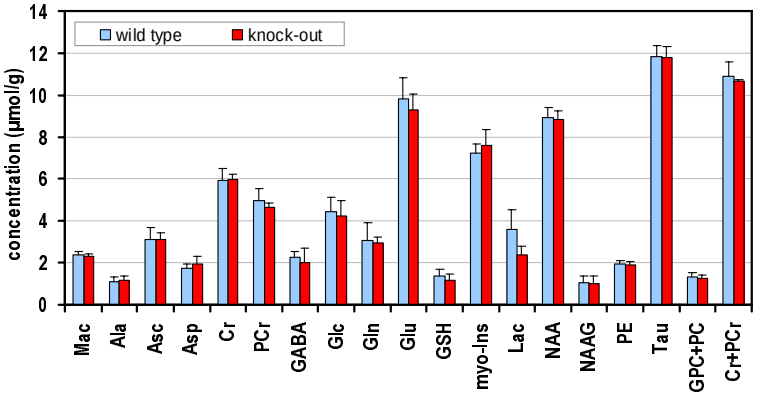

Fig. Neurochemical profiles of hippocampal-specific iron deficient KO mice and WT controls. Postnatal age 45, n = 6, Error bars = SD.

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