Research Article

NMR IN BIOMEDICINE

Received: 10 July 2009, Revised: 12 October 2009, Accepted: 5 December 2009, Published online in Wiley InterScience: 16 March 2010

(www.interscience.wiley.com) DOI:10.1002/nbm.1498

Neurochemical profile of the mouse hypothalamus using *in vivo* ^{1}H MRS at 14.1T

Hongxia Lei[a,b]*, Carol Poitry-Yamate[a], Frédéric Preitner[c], Bernard Thorens[c] and Rolf Gruetter[a,b,d]

The hypothalamus plays an essential role in the central nervous system of mammals by among others regulating glucose homeostasis, food intake, temperature, and to some extent blood pressure. Assessments of hypothalamic metabolism using, e.g. ^{1}H MRS in mouse models can provide important insights into its function. To date, direct *in vivo* ^{1}H MRS measurements of hypothalamus have not been reported. Here, we report that *in vivo* single voxel measurements of mouse hypothalamus are feasible using ^{1}H MRS at 14.1T. Localized ^{1}H MR spectra from hypothalamus were obtained unilaterally (2–2.2 μL, VOI) and bilaterally (4–4.4 μL) with a quality comparable to that of hippocampus (3–3.5 μL). Using LCModel, a neurochemical profile consisting of 21 metabolites was quantified for both hypothalamus and hippocampus with most of the Cramér-Rao lower bounds within 20%. Relative to the hippocampus, the hypothalamus was characterized by high γ-aminobutryric acid and *myo*-inositol, and low taurine concentrations. When studying transgenic mice with no glucose transporter isoform 8 expressed, small metabolic changes were observed, yet glucose homeostasis was well maintained. We conclude that a specific neurochemical profile of mouse hypothalamus can be measured by ^{1}H MRS which will allow identifying and following metabolic alterations longitudinally in the hypothalamus of genetic modified models. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: ^{1}H MRS; neurochemical profile; hypothalamus; high magnetic fields; mouse; glucose transporter

INTRODUCTION

The hypothalamus has been intensively studied because of its important role in the central nervous system linking the central nervous system to the endocrine system via the pituitary gland, such as co-ordinating hormonal and behavioral circadian rhythms, neuroendocrine regulation, and governing emotional behavior ((1,2) and references therein). In addition, the orchestration and control of glucose and energy homoeostasis by the hypothalamus makes this brain region central to physiological and metabolic studies ((3) and references therein). The use of experimental models, particularly transgenic mouse models, is of great interest for elucidating the multiple functions of the hypothalamus and stands to benefit from non-invasive *in vivo* studies.

MRI and MRS have been shown to be powerful investigation tools for non-invasive studies. In particular, ^{1}H MRS has been shown capable of measuring a neurochemical profile consisting of 18 metabolites including neurotransmitters and energy substrates from mouse striatum, cortex, thalamus, hippocampus and cerebellum (4), but the measurement of smaller and deep lying structures, such as the hypothalamus, has not yet been reported. Both the location of the hypothalamus in the brain and its small volume represent formidable challenges for ^{1}H MRS.

High magnetic fields increases sensitivity, spectral resolution and consequently lead to lower Cramér-Rao lower bounds (CRLBs) (5). For example, the intrinsically increased sensitivity allowed detecting progressively lowered spectral signal intensities due to ischemia-induced cellular death in mouse striatum (6).

In this study, we report that such technical improvements allowed *in vivo*, single voxel measurements of the neurochemical profile from mouse hypothalamus at 14.1T. Furthermore, we

* *Correspondence to: H. Lei, Laboratory of Functional and Metabolic Imaging (LIFMET), Institute of the Physics of Biological Systems, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switzerland.*
E-mail: hongxia.lei@epfl.ch

a *H. Lei, C. Poitry-Yamate, R. Gruetter*
Laboratory of Functional and Metabolic Imaging (LIFMET), Institute of the Physics of Biological Systems, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

b *H. Lei, R. Gruetter*
Department of Radiology, University of Lausanne, Lausanne, Switzerland

c *F. Preitner, B. Thorens*
Center for Integrative Genomics, University of Lausanne, Lausanne, Switzerland

d *R. Gruetter*
Department of Radiology, University of Geneva, Geneva, Switzerland

Contract/grant sponsor: Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV and EPFL.
Contract/grant sponsor: The Swiss National Science Foundation; contract/grant number: 31003A-113525.
Contract/grant sponsor: The Leenaards and Jeantet Foundations.

Abbreviations used: *Ala, alanine; Asc, ascorbate; Asp, aspartate; Cr, creatine; CRLB, Cramér-Rao lower bound; myo-Ins, myo-inositol; GABA, γ-aminobutryric acid; Glc, glucose; Gln, glutamine; Glu, glutamate; GLUT, glucose transporter; GLUT8, glucose transporter isoform 8; Gly, glycine; GPC, glycerophosphocholine; GSH, glutathione; KO, knockout; Lac, lactate; Mac, macromolecule; NAA, N-acetyl-aspartate; NAAG, N-acetyl-aspartyl-glutamate; PCho, phosphocholine; PCr, phosphocreatine; PE, phosphorylethanolamine; Scyllo, scyllo-inositol; Tau, taurine; VOI, volume of interest.*

examined quantitatively the effect of deleting the glucose transporter protein isoform 8 (GLUT8) on the neurochemical constituents including glucose of both hippocampus and hypothalamus, in both of which GLUT8 is normally expressed and highly involved (7,8).

METHODS

Animal protocols

Three groups of mice were studied with approval by the local ethics committee. In the first group, eight adult C57BL/6 male mice (29 ± 4 g, Charles River, L'Arbresle Cedex, France) were studied to evaluate the feasibility of ^{1}H MRS measurements of hypothalamus, as described below. GLUT8 knockout mice (GLUT8 KO) were backcrossed in the C57BL6/J@RJ background, which were originally from Janvier, le Genest St. Isle, France, thereafter internally bred at the Center for Integrative Genomics, University of Lausanne, Switzerland. Six GLUT8 KO mice and six control mice were subjected to the MR measurements at age of 12–14 weeks.

For MR studies, anesthesia was induced with 3% isoflurane mixed with O_2 and thereafter maintained with $0.8 \sim 1.5\%$ isoflurane. Right after stereotaxic fixation in a holder (RAPID Biomedical GmbH, Rimpar, Gemany), the animal underwent MR studies while breathing patterns and temperature were monitored through a MR-compatible system (Model 1025, SA Instruments Inc., Stony Brook, NY, US). Rectal temperature was maintained at $\sim$36°C by circulating warm water.

Magnetic Resonance instruments

All MR studies were carried out in a horizontal, 14.1T/26 cm magnet (Magnex Scientific, Abingdon, UK), with a 12-cm-inner-diameter gradient (400 mT/m in 200 μs), and 2nd order shim coils with maximum strengths of $Z^2 = 5.3 \times 10^{-2}$mT/cm^2, $YZ = 1.2 \times 10^{-1}$mT/cm^2, $XZ = 1.2 \times 10^{-1}$mT/cm^2, $XY = 4.5 \times 10^{-2}$mT/cm^2 and $X^2Y^2 = 4.2 \times 10^{-2}$mT/cm^2 (Varian/Magnex Scientific, UK), interfaced to a DirectDrive console (Varian Inc., Palo Alto, CA, US). A home-built quadrature surface coil with two geometrically decoupled single-turn loops (12 mm inner-diameter) resonating at 600-MHz radio frequency (RF) was used as RF transceiver (9).

For localization and identification of anatomical landmarks, multi-slice T_2-weighted images were acquired using the fast spin echo (FSE) technique (10) with a field of view of 20×20 mm^2, 256^2 data matrix, 0.6-mm slice thickness, effective TE = 50 ms and TR = 6000 ms. Thereafter, all 1st and 2nd order shim terms were adjusted over the respective VOI using the echo-planar version of FASTMAP (11), which resulted in water linewidths within 25 Hz. Localized ^{1}H MR spectra were acquired using SPECIAL (12), using TE/TR = 2.8/4,000 ms in combination with outer volume suppression and VAPOR water suppression (13). To obtain adquate signal-to-noise ratio (SNR), 320 or 480 scans were acquired for unilateral hippocampus or bilateral hypothalamus, respectively. To assess the potential partial volume effect of the 3rd ventricle on measuring the neurochemical profile from the bilateral hypothalamus, we simultaneously measured localized MR spectra of unilateral hypothalamus ($1 \times 1 \times 2$–$1 \times 1.2 \times 2.2$ mm^3) in five of the eight mice using 640 averages. For absolute quantification, water signal was acquired using identical parameters, except without water suppression and averaging eight scans.

Quantification and statistics

The *in vivo* ^{1}H MR spectra obtained were processed as described previously (4). A linear combination analysis method, LCModel (14) was used to calculate metabolite concentrations from a fit to the experimental spectrum based on known spectra of metabolites (basis set), in combination with referencing to the corresponding endogenous water signal (100% visibility and molarities of 55.5 mol/L) (15). Absolute quantification was accomplished assuming 80% brain water content, which allowed comparison to previous studies performed under similar assumptions and conditions (4). In this study, all metabolites except macromolecules (Mac) in the basis set of the LCModel were simulated, i.e. alanine (Ala), ascorbate (Asc), aspartate (Asp), creatine (Cr), *myo*-inositol (*myo*-Ins), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycine (Gly), glycerophoshocholine (GPC), glutathione (GSH), lactate (Lac), N-acetyl-aspartate (NAA), N-acetyl-aspartyl-glutamate (NAAG), phosphocholine (PCho), phosphocreatine (PCr), phosphorylethanolamine (PE), scyllo-inositol (Scyllo), and taurine (Tau). Mac was measured in a volume including hypothalamus of one mouse (C57BL/6) using an inversion recovery technique (TE/TI/TR = 2.8/750/2500 ms), processed and included in the basis set as described previously (5). Thereafter, Mac was assumed to have minimal pattern difference between either regions or strains. Measurements with CRLB > 50% were considered not detectable. Since GPC and PCho were strongly correlated in the LCModel analysis ($r = -0.8$), the sum of GPC and PCho was evaluated.

All data are presented as mean ± SD. Two-tailed student *t*-tests were applied to perform statistical analysis. In particular, a paired *t*-test was applied for comparing data acquired from different brain regions in the same animals, and for comparing the difference between unilateral and bilateral hypothalamus. To correct for multiple comparisons, *p*-value thresholds $p < 0.01$ (*), $p < 0.002$ (**), $p < 0.0002$(***) were used to determine statistical significance. Based on Bonferroni correction, the latter two p value thresholds were considered suitable for 25 comparisons.

RESULTS

Multi-slice FSE T_2-weighted images at 14.1T provided adequate spatial resolution and anatomical contrast for accurate positioning of the VOIs, in particular in the hippocampus and the hypothalamus based on anatomical landmarks (Fig. 1). The field inhomogeneities were compensated locally without exceeding 50% of the maximum strengths of the supplied 2nd order shim coils, i.e. 47% in hypothalamus ($Z^2 \leq 1.5 \times 10^{-2}$mT/cm^2, $YZ \leq 1.9 \times 10^{-2}$mT/cm^2, $XZ \leq 5.6 \times 10^{-2}$mT/cm^2, $XY \leq 1.3 \times 10^{-2}$mT/cm^2 and $X^2Y^2 \leq 2.0 \times 10^{-2}$mT/cm^2) and 34% in hippocampus ($Z^2 \leq 1.6 \times 10^{-2}$mT/cm^2, $YZ \leq 2.1 \times 10^{-2}$mT/cm^2, $XZ \leq 3.6 \times 10^{-2}$mT/cm^2, $XY \leq 1.3 \times 10^{-2}$mT/cm^2 and $X^2Y^2 \leq 1.4 \times 10^{-2}$mT/cm^2). VAPOR water suppression resulted in a residual water signal below NAA and total creatine (Cr + PCr). As a result, ^{1}H MR spectra of hypothalamus were obtained with metabolite linewidths of 17 ± 2Hz along with extracerebral lipid contamination at 1.3–1.7 ppm effectively eliminated, and comparable to those of hippocampus, measured with metabolite linewidths of 16 ± 3Hz (Fig. 1). The corresponding SNRs of the spectra from hypothalamus were 14 ± 1, similar to those from hippocampus, i.e. 14 ± 4.

When comparing the ^{1}H MR spectra of hypothalamus with those of hippocampus, striking differences were apparent, such as the elevated GABA and GPC + PCho peaks, and the lower Ala, Glu, Tau, *myo*-Ins and PCr + Cr signal intensities (Fig. 1). LCModel

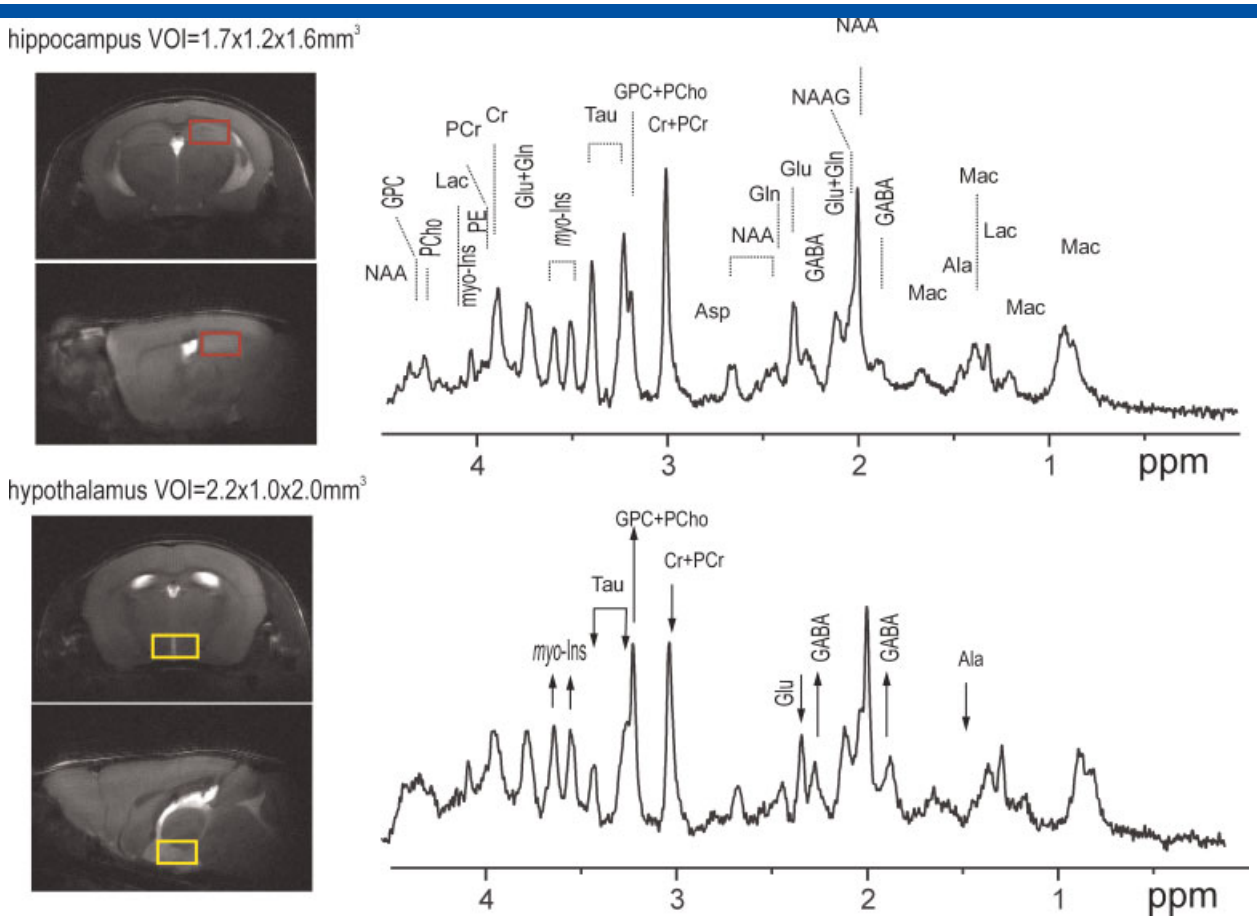

Figure 1. Typical multi-slice FSE images and localized ^{1}H MR spectra of hippocampus and hypothalamus at 14T in one mouse. Spectra (TE/TR = 2.8/4000 msec) were obtained from the indicated VOIs (marked red for hippocampus (NT = 320) and yellow for hypothalamus (NT = 480)), and shown with Gaussian apodization (gf = 0.15 sec). Relative to the hippocampus, the striking spectral features of hypothalamus are indicated with the oriented arrows along the corresponding metabolites.

quantification of the ^{1}H MR spectra from the hippocampus resulted in 21 individual metabolite concentrations with minimal fit residuals, extending the previously reported number of metabolites (4) to the addition of Asc, Asp (in five out of eight animals), Gly and Scyllo (Fig. 2). Most metabolite concentrations were similar to previous results (4), but reduced CRLBs. In addition, choline compounds, GPC and PCho, were quantified separately with CRLBs mostly within 35%. Likewise, the neurochemical profile of bilateral hypothalamus was obtained where nearly all metabolites, including GPC, were quantified with CRLBs $< 20\%$ and mostly with CRLBs $< 15\%$. Due to the comparatively decreased concentrations in hypothalamus, Ala and Scyllo were quantified in four animals with CRLBs $< 50\%$ (Fig. 2). Across all metabolite concentrations in hypothalamus, the average CRLBs was no more than 0.35 μmol/g (Fig. 2). Relative to the hippocampus, the neurochemical profile of hypothalamus had significantly lower Ala, Cr, Glu, NAA, PCr and Tau contents, and increased GABA, GPC, GPC + PCho, *myo*-Ins and PE concentrations (Fig. 2). After correcting for multiple comparisons, regional differences in the neurochemical profile of hypothalamus were found in Ala, Cr, GABA, Glu, GPC, GPC + PCho, *myo*-Ins, NAA, PE and Tau, in agreement with the apparent signal difference visually observed in the spectra (Fig. 1).

To determine whether potential partial volume effects from the 3rd ventricle [~10% reported by Ma *et al.* 2008 (16)] may affect absolute quantification, ^{1}H MR spectra of unilateral hypothalamus were measured. The ^{1}H MRS measurement of unilateral hypothalamus excluding the 3rd ventricle did not present any discernable signal discrepancy or quality reduction in spectra when compared with spectra of bilateral hypothalamus from the same animal (Fig. 3a). The resulting average metabolic linewidth of 17 ± 1Hz from unilateral hypothalamus was nearly identical to that from bilateral hypothalamus, i.e. 17 ± 2Hz. By increasing the number of averages, the SNR was 10 ± 1 and remained sufficient to measure nearly all metabolites quantifiable within CRLBs $< 50\%$ except for Ala and Scyllo. Concentrations obtained from unilateral hypothalamus were not different from those of bilateral hypothalamus (Fig. 3). In particular, NAA and Mac were unaltered (Fig. 3b). Of the highly concentrated metabolites, only Tau and the sum of Cr and PCr were increased by more than 10% with $p = 0.01$ and $p = 0.03$ respectively.

In the animals with deletion of GLUT8, we noted that most metabolites were unaffected ($p > 0.05$, Fig. 4) when compared to their wild-type counterparts (unpaired student *t*-test), with the exception of a slight increase in the total of PCr and Cr ($p = 0.02$) and Tau ($p = 0.04$) in hippocampus and a small reduction of Gln in hypothalamus ($p = 0.02$).

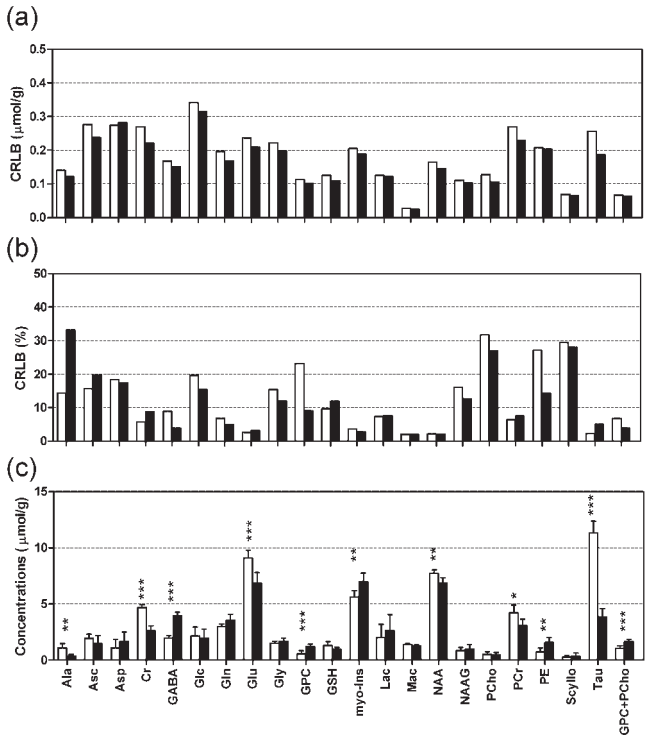

Figure 2. Neurochemical profiles of mouse hypothalamus and hippocampus. In (a), the neurochemical profiles of hippocampus (white bars) and hypothalamus (solid bars) are shown along with the observed significant differences ('*'$p < 0.01$, '***'$p < 0.002$ and '***'$p < 0.0002$ (paired student *t*-test)). The corresponding CRLBs for each metabolite are shown in % [panel (b)] and μmol/g [Panel (c)] respectively.

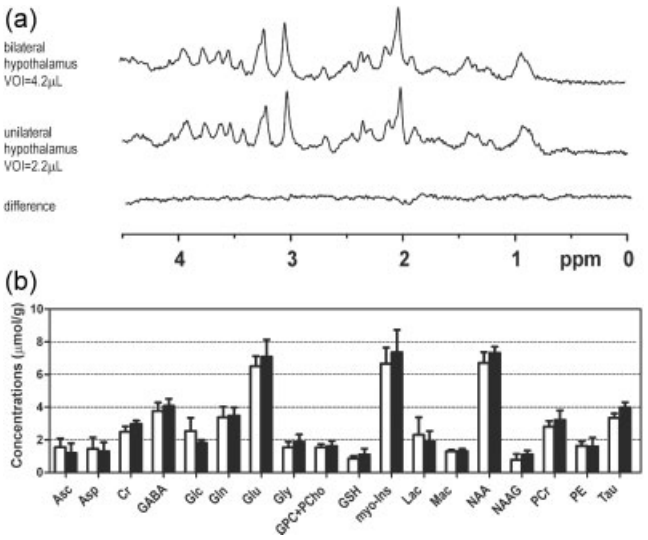

Figure 3. Comparison of spectra and selected metabolites measured bilaterally (white bars) and unilaterally hypothalamus (black bars) in mouse hypothalamus. In (a), bilateral (VOI = 4.2 μL, 420 scans) and unilateral (VOI = 2.2 μL, 680 scans) MR spectra from one mouse hypothalamus were compared. Both spectra were processed to achieve an identical linewidth by Gaussian apodization (gf = 0.08 sec). No apparent difference was visually observed in the spectra (a) and thereafter confirmed by direct subtraction of the spectrum from the bilateral hypothalamus from that of the unilateral hypothalamus [the trace labeled as 'difference' in (a)]. In (b), comparison of the neurochemical profile was displayed between uni- (black bars) and bilateral hypothalamus (white bars).

DISCUSSION

In this study, high quality spectra (high spectral resolution, sufficient SNR, excellent localization performance, efficient water suppression) were reproducibly obtained from mouse hypothalamus. This was achieved despite challenges due to the small mouse brain itself and due to the distant location of the hypothalamus. Nearly 21 metabolites were reliably quantified and thus substantial metabolic information of mouse hypothalamus was obtained *in vivo*. To our knowledge, this study is the first *in vivo*, single voxel measurement of the neurochemical profile of mouse hypothalamus.

With increased magnetic field strength, the intrinsically amplified susceptibility differences in mouse brain between surrounding air and tissue can be minimized by means of automatic shimming methods (11), especially important for hypothalamus where nearly identical metabolic linewidths were achieved as those in hippocampus without exceeding any shim strength limitations (see Results). The resulting linewidths did not change with the size of VOIs and were slightly broader than previous rat studies (5) but within the reported range of mouse striatum (6) at the same field strength, which could be explained by either limitations of 3rd or higher order shim terms or by possible physiological contributions. Nonetheless, a very high spectral resolution in both hippocampus and hypothalamus was reproducibly accomplished as is illustrated in Figure 1.

Along with the increased distance of the hypothalamus from the surface RF coil used here relative to that of the hippocampus, the reduced sensitivity was partially compensated by means of using a spin-echo full intensity localizing technique and by slightly increasing total scan time. The resulting SNRs from bilateral hypothalamus (4–4.4 μL) in a reasonable measuring time (30 min) were nearly identical to those from unilateral hippocampus (~3 μL) acquired in 20 min. The sensitivity at 14.1T

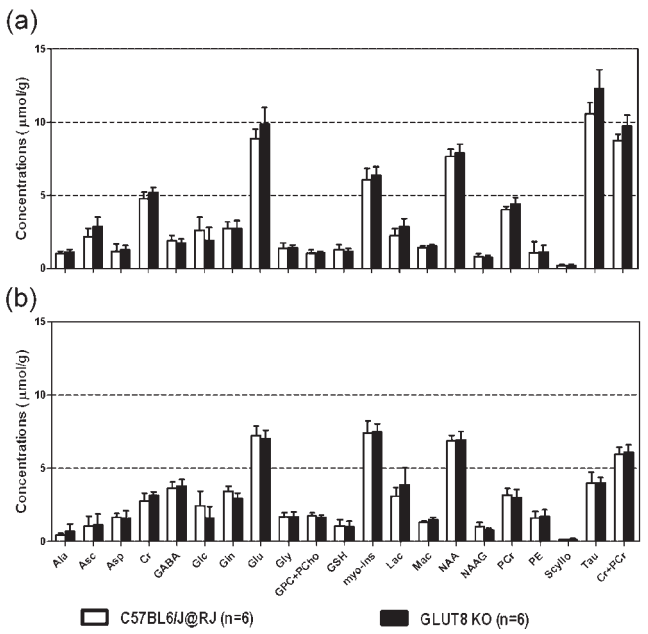

Figure 4. The neurochemical profile of hippocampus (a) and hypothalamus (b) in GLUT8 KO (black bars) and wild type mice (C57BL6/J@RJ, white bars). Error bars are SDs.

provided SNR ~14 from both VOIs (Fig. 2). Even with 40-min acquisition time, the resulting SNRs of the MR spectra acquired from the *unilateral* hypothalamus (2–2.4 μL) remained sufficient for absolute quantification of most metabolites, as in previous studies (6). It is of interest to note, that for ^{1}H MRS at, e.g. 9.4T (4), the estimated 50% reduced sensitivity compared to 14.1T (5) can be compensated by approximately doubling the total scan time, which may not be practical for many applications.

In all measurements, characteristic features of hypothalamic metabolite concentrations were observed relative to those in hippocampus, such as high concentrations of myo-Ins and GABA and low Tau content. None of these concentration differences would be explained by the presence of altered concentrations of other compounds, such as Mac, since it was highly unlikely for Mac to contribute all spectral changes in both Tau and GABA (Fig. 1). In addition, these hallmarks of hypothalamic concentrations were consistent with previously reported biochemical values in rats (17) and mice (18,19), in particular the high GABA and low Glu concentrations, which could point to GABA as a dominant neurotransmitter in hypothalamus (20). Furthermore, the observed increased hypothalamic choline content mostly in the form of GPC was in excellent agreement with chromatographic measurements in mice (21).

When comparing bilateral to unilateral hypothalamus, we found that the metabolite concentrations were highly consistent (Fig. 3). This suggested that measuring bilateral hypothalamus can be achieved with minimal partial volume effects from CSF in the 3rd ventricle. For example, when assuming a 10–25% of CSF volume in bilateral hypothalamus VOI (16), the water content used to quantify metabolites should increase only by 2–5% since normal brain already has ~80% water content. Therefore, the concentrations would increase by 2–5%, which will not change the main conclusion of the present study that a neurochemical profile specific to the unilateral hypothalamus can be measured *in vivo*.

It is interesting to note that the Glc content measured in hypothalamus was nearly identical to that in hippocampus (Fig. 2), suggesting that glucose homeostasis in hypothalamus was similar to that of the hippocampus. This is consistent with the presence of an intact blood–brain barrier, in agreement with a recent biochemical study in rats (22).

Animals devoid of GLUT8 did not present any significant changes when compared with the age-matched wild-type controls suggesting that glucose homeostasis was well-maintained in hippocampus and hypothalamus of GLUT8 KO animals (Fig. 4). Nonetheless, small changes in the observed neurochemical profiles of GLUT8 KO mice were found in the sum of PCr and Cr, and Tau in hippocampus, and hypothalamic Gln. Therefore, the measured 50% increase of cell proliferation in hippocampus in GLUT8 KO mice at the same age (7) could be a possible explanation for retaining the apparent normal neurochemical profile and similar glucose contents.

In the present study we assumed that the signal from Mac did not vary substantially from region to region or due to genetic modifications. The assumed unaltered patterns of Mac was further supported by LCModel analysis from the minimal fit residuals, as demonstrated in Results. In addition, the attributes of hypothalamus relative to the hippocampus, such as low Tau and high GABA contents as determined by LCModel were visually striking in the MR spectra (Fig. 1). Nonetheless, the main conclusion of the present paper, namely that assessing the neurochemical profile of mouse hypothalamus *in vivo* using ^{1}H MRS is feasible, is not affected by these considerations.

We conclude that localized ^{1}H MRS of mouse hypothalamus *in vivo* is feasible at high magnetic fields. This novel technique opens new possibilities to study this important brain region in genetic models of disease.

Acknowledgements

The work was supported by the Centre d'Imagerie BioMédicale (CIBM) of the UNIL, UNIGE, HUG, CHUV and EPFL, the Swiss National Science Foundation 31003A–113525, and the Leenaards and Jeantet Foundations.

The authors appreciated helpful discussion with Dr Vladimir Mlynarik.

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