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Determination of Oxidative Glucose Metabolism *in vivo* in the Young Rat Brain using Localized Direct-detected ^{13}C NMR Spectroscopy

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Abstract

Determination of oxidative metabolism in the brain using *in vivo* ^{13}C NMR spectroscopy (^{13}C MRS) typically requires repeated blood sampling throughout the study to measure blood glucose concentration and fractional enrichment (input function). However, drawing blood from small animals, such as young rats, placed deep inside the magnet is technically difficult due to their small total blood volume. In the present study, a custom-built animal holder enabled temporary removal of the animal from the magnet for blood collection, followed by accurate repositioning in the exact presampling position without degradation of B_0 shimming. ^{13}C label incorporation into glutamate C4 and C3 positions during a 120 min $[1,6-^{13}\text{C}_2]$ glucose infusion was determined in 28-day-old rats ($n = 4$) under α -chloralose sedation using localized, direct-detected *in vivo* ^{13}C MRS at 9.4T. The tricarboxylic acid cycle activity rate (V_{TCA}) determined using a one-compartment metabolic modeling was $0.67 \pm 0.13 \mu\text{mol/g/min}$, a value comparable to previous *ex vivo* studies. This methodology opens the avenue for *in vivo* measurements of brain metabolic rates using ^{13}C MRS in small animals.

Keywords

Brain; ^{13}C NMR spectroscopy; development; glucose; oxidative metabolism; rat

Introduction

As in the mature brain, glucose is the principal energy substrate for the developing human and rat brains. In both species, the cerebral glucose transport and utilization increases rapidly after birth, paralleling the structural and functional brain maturation [1–3]. Most of the transported glucose is oxidatively metabolized through the tricarboxylic acid (TCA) cycle in the brain [2].

^{13}C NMR spectroscopy (^{13}C MRS) combined with metabolic modeling is an established method for assessing oxidative glucose metabolism in the adult human and animal brains *in vivo* [4–8]. In this method, ^{13}C -enriched glucose, typically labeled at C1 and/or C6

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Conflicts of interest

The authors declare that they have no conflict of interest.

positions, is intravenously infused and the dynamic incorporation of ^{13}C label in cerebral metabolites is monitored using *in vivo* MRS. The TCA cycle rate (V_{TCA}) is determined using metabolic modeling (Reviewed in [9–11]).

Thus far, ^{13}C NMR studies in small rodents, such young rats, have been limited to *ex vivo* analysis of the brain tissue [12–15]. In this method, after administering labeled glucose, small groups of animals are killed at predetermined intervals for determining ^{13}C label incorporation in brain metabolites. While such studies permit assessment in conscious animals and have provided important insights into glucose metabolism during normal development and in neuropathologies, they require large sample sizes, and the results may be confounded by inter-animal variations, tissue preparation [14] and postmortem extraction artifacts [9,10]. *In vivo* ^{13}C MRS overcomes these problems by tracking the entire time course of ^{13}C label incorporation in the same animal.

Even when *in vivo* ^{13}C MRS has been possible in small rodents [16], metabolic modeling has remained a challenge. Metabolic modeling typically requires frequent blood sampling throughout the study to measure plasma glucose concentration and ^{13}C fractional enrichment (FE) (input function). When animals are placed deep inside the magnet, long vascular catheters are necessary for bringing the blood to the opening of the magnet bore for collection. Whereas the actual blood volume required for analysis is small (0.1–0.2 ml per sample), a much larger volume (typically, 1 ml) is necessary to fill the catheters (“dead volume”), which is subsequently returned to the animal. While such a strategy is feasible in adult rats with a total blood volume of ~20 ml, frequent drawing of 1–1.5 ml of blood is not possible in small rodents because of their small total blood volume. The difficulty in measuring the input function has hindered the application of *in vivo* ^{13}C MRS and metabolic modeling in small rodents.

In a recent study, Valette et al., showed that metabolic modeling is possible without the knowledge of input function, provided that steady-state plasma FE is achieved rapidly with the infusion and remains constant for the duration of the experiment [17]. However, unlike in bigger animals or humans, it is difficult to achieve such a stable fractional enrichment in small rodents, and the reliable knowledge of the exact input function in each individual animal becomes necessary for modeling.

In the present study, we measured the glucose metabolic rates in the brain of 28-day-old rats using *in vivo* ^{13}C MRS. Rats of this age have yet to achieve physical, sexual and neurodevelopmental maturity and are used to model neurological disorders of young children [18–20]. The body weight at this age is approximately 25%–33% of adult rats (100 g vs. 350–450 g) and the total blood volume is approximately 6–8 ml [21]. We overcame the difficulties with blood collection by designing a custom-built animal holder that enabled removal of the animal temporarily from the magnet for blood collection, followed by repositioning in the exact presampling location with high degree of accuracy and reproducibility. This allowed us to briefly remove the rats from the magnet for blood sampling with negligible impact on voxel position and B_0 shimming. Our aim was to establish the feasibility and reliability of this approach for the *in vivo* assessment of V_{TCA} in small animals.

Experimental Procedure

Animal Preparation

The Institutional Animal Care and Use Committee approved all experiments. 28-day-old Sprague-Dawley rats ($n = 4$; 102 ± 8 gm, Harlan, Indianapolis, IN, U.S.A.) were studied after overnight fasting. Rats were intubated and mechanically ventilated (Kent Scientific,

Litchfield, CT, U.S.A.) as previously described [22]. One femoral artery and both femoral veins were cannulated using polyurethane catheters (1.2F; Instech Solomon, Plymouth Meeting, PA, U.S.A.). The venous catheters were >5 m in length and were used for infusion of glucose and α -chloralose. The arterial catheter was approximately 30 cm long and was used for blood draws. Isoflurane was discontinued and light anesthesia using α -chloralose (40 mg/kg bolus, followed by continuous infusion at 26.8 mg/kg/h) was used for the remainder of the study. Ventilator settings were adjusted based on arterial blood gas and end tidal CO₂ [22]. The rectal temperature was maintained at 37°C using temperature probe (Cole Palmer, Vernon Hills, IL, U.S.A.) and thermosensor-regulated warm water circulating in tubes (RTE-101 bath calculator, Thermo NESLAB, Portsmouth, NH, U.S.A.).

Animal holder

A custom-designed animal holder was used (Figure 1). The animal holder and the mounting head functioned as a lock and key mechanism that allowed fine rotational adjustment (range: ± 20 degrees). The rat was placed prone in a cradle and the head was secured using bite-bar and ear rods as in our previous studies [23,24]. The cradle with the rat was placed inside the animal holder and secured using screws. The surface RF coil was secured over the head. The holder with the rat was placed inside the magnet and scout images were obtained. The optimal rotation angle was established by manually rotating the inner ring of the mounting head (Figure 1). This rotational position was secured using the rotation-tightening knobs and maintained for the duration of the experiment. The anterior-posterior (translational) position was adjusted using the translation adjustment knob on the holder while the translatable locking pin on the holder was anchored in the locking pin bracket on the mounting head and secured using translation-tightening knobs (Figure 1). Releasing the locking pin bracket and loosening the translation-tightening knobs allowed withdrawal of the animal holder from the magnet for blood draws. Replacing the holder and locking the translation pin inside the bracket guaranteed repositioning of the head in the exact presampling position with high degree of accuracy and reproducibility.

Glucose Infusion Protocol

Plasma glucose concentration was rapidly raised to hyperglycemia levels [plasma glucose >13.8 mmol/L (>250 mg/dl)] using published protocol [24]. Briefly, a bolus of 99%-enriched [1,6-¹³C₂] glucose (20% wt/vol; Isotec, St. Louis, MO, U.S.A.) was infused over 5 min with the infusion rate decaying 50% each successive min, followed by continuous infusion of 70%-enriched glucose (20% wt/vol) for the remainder of the experiment [24]. Arterial blood samples were collected at 5, 10, 20, 30, 60, 90 and 120 min after the start of infusion for determining blood gases, plasma glucose levels and ¹³C FE. For this, the animal holder was withdrawn from the magnet until the indwelling arterial catheter could be accessed. Approximately 150 μ l of blood was collected without disturbing the surface RF coil and the position of the rat. After flushing the catheter with heparinized normal saline, the animal holder was replaced inside the magnet and secured in the pre-sampling position. Each instance of removal from the magnet, blood collection and repositioning required approximately 2 min. Plasma glucose was determined using the glucose oxidase method (Model GM7 Micro-stat; Analox Instruments, London, United Kingdom). Minor adjustments were made to the infusion rates to maintain plasma glucose between 13.8 mmol/L and 18 mmol/L (250–325 mg/dl). Plasma ¹³C FE was determined using the gas chromatography-mass spectrometry method using published methods [8,25].

In vivo ¹³C NMR Spectroscopy

In vivo NMR spectra were acquired from all four rats using a 9.4T/31 cm horizontal bore magnet interfaced to an Inova console (Varian, Palo Alto, CA, U.S.A.). The magnet was equipped with gradients reaching 450 mT/m in 200 μ s (Resonance Research Inc, Bilerica,

MA). The RF coil assembly consisted of a ^1H quadrature surface coil (two loops of 14 mm diameter) and an inner ^{13}C linearly polarized surface coil (12 mm diameter). Fast spin-echo (FSE) images (echo train length = 8, echo spacing = 15 ms, echo time = 48 ms, field of view = $2.5\text{ cm} \times 2.5\text{ cm}$, matrix = 256×256 , slice thickness = 1 mm) were acquired to select an $8 \times 5 \times 9\text{ mm}^3$ volume of interest (VOI) within the brain. NMR spectra were obtained as described previously [24]. Briefly, all first and second-order shims were adjusted using FAST(EST)MAP, which resulted in an average ^1H water linewidth of 22 Hz in the $360\text{ }\mu\text{l}$ VOI. Localized *in vivo* ^{13}C NMR spectra were acquired using a semiadiabatic distortionless enhancement by polarization transfer (DEPT) sequence [24,8]. Localization was performed on the ^1H magnetization using 3D-ISIS combined with outer volume suppression (OVS). The inter-pulse delay was 3.8 ms (optimized for $J_{\text{CH}} = 130\text{ Hz}$) and the nominal flip angle of the last ^1H pulse was set to 45° in order to simultaneously detect signals from CH, CH_2 , and CH_3 groups. Repetition time was 2.5 s. ^1H decoupling with WALTZ-16 composite was applied during the acquisition time (205 ms, 8192 complex points) with a nominal $\gamma B_1/2\pi$ of 2 kHz. Data were collected in blocks of 128 scans resulting in a temporal resolution of 5.3 min.

Additionally, to confirm that the procedure for blood collection did not impact the rat's position inside the magnet, FSE images were obtained in sagittal and coronal planes before and after four instances of removal and repositioning inside the magnet in a 28-day-old rat.

Ex vivo ^{13}C NMR Spectroscopy of Brain Extracts

In vivo ^{13}C time courses were scaled using FE measured in the brain extracts collected at the end of the experiment. At the completion of *in vivo* MRS, rats were killed using focused microwave fixation (4kW within 1.0 sec; Gerling Applied Engineering, Modesto, CA, U.S.A.). The entire brain, excluding cerebellum was removed and digested in 3 ml ice-cold perchloric acid (0.9M). Tissue extracts were prepared as described previously [26]. Briefly, digested brain tissue was centrifuged for 15 min at 4°C . The supernatant was neutralized with 9M KOH. Metallic ions were removed by adding resin (Chelex®100; Bio-Rad Laboratories, Hercules, CA, U.S.A.) to the supernatant, which was then filtered, freeze-dried and resuspended in 10:90 $\text{D}_2\text{O}:\text{H}_2\text{O}$ solution ($\text{pH } 7.2 \pm 0.1$).

The brain extracts were analyzed using ^{13}C MRS at 14.1T (Varian Unity Inova spectrometer). One-dimensional pulse-acquired ^{13}C spectra were acquired with Nuclear Overhauser Effect (NOE) enhancement under fully relaxed conditions ($\text{TR}=15\text{ s}$, spectral width 30,000 Hz, acquisition time 1 sec and ^1H decoupling with WALTZ-16). Comparison of spectra acquired with and without NOE showed that NOE enhancement was identical for Glu-C4 and Glu-C3, and therefore no correction was applied for NOE. The built-in spectrometer software was used to fit resonances after zero-filling to 512K points and resonances were assigned based on peak position using published values [26]. All chemical-shift values were referenced to lactate (Lac, 21 ppm).

LCModel Analysis

Metabolite concentrations in the *in vivo* ^{13}C spectra were determined using LCModel (Provencher, Oakville, Ontario, Canada) [26,8]. No baseline correction, zero-filling, or time domain filtering were applied prior to LCModel analysis. Basis spectra for metabolites were simulated using a custom-written program (Matlab; The MathWorks, Natick, MA, U.S.A.) including ^{13}C - ^{13}C J-modulation. The relative ^{13}C concentrations determined by LCModel were converted to absolute ^{13}C concentrations using the isotopic enrichments measured in the brain extracts at the end point and assuming a glutamate (Glu) concentration of $10\text{ }\mu\text{mol/g}$, based on previous ^1H MRS data from 28-day-old rats [27].

Metabolic Modeling

Time courses of ^{13}C -label incorporation into the Glu-C4 and Glu-C3 positions were analyzed using the one-compartment model using SAAM II software (University of Washington, WA, U.S.A.) [8]. In each rat, its own individual dynamic FE time course of plasma glucose was used as input function to fit the *in vivo* data. In addition to V_{TCA} , V_{X} (exchange rate between α -ketoglutarate and Glu) and V_{OUT} (dilution of labeled ^{13}C -Lac through exchange with unlabeled Lac) were included as unknown (free) parameters in the fit. To account for the loss of ^{13}C -labeled glutamine (Gln) through exchange with unlabeled Gln, another fitted parameter (V_{EFF}) was added for dilution of Gln. The following concentrations were assumed in the model based on previous studies [5, 27]: $[\text{Glu}] = 10 \mu\text{mol/g}$, $[\text{Gln}] = 2.5 \mu\text{mol/g}$, $[\text{oxaloacetate}] = 1 \mu\text{mol/g}$, $[\text{Lac}] = 1 \mu\text{mol/g}$. Finally, the following fluxes were assumed in the model based on a previous study [5]: V_{GLN} (rate of Gln synthesis) $= 0.41 \times V_{\text{TCA}}$ and V_{GLY} (rate of glycolysis) $= 0.5 \times V_{\text{TCA}}$.

All data are presented as mean $\pm$ SEM.

Results

All the rats were in stable physiological conditions during the experiment. The arterial blood gas was within normal range: pH, 7.32 ± 0.01 , PaO_2 , $143.2 \pm 7.0 \text{ mm Hg}$ and PaCO_2 , $37.3 \pm 1.5 \text{ mm Hg}$. The rectal temperature was maintained at $36.7 \pm 0.2^\circ\text{C}$.

Rats were repeatedly removed from the magnet for blood sampling. The custom-designed animal holder ensured repositioning of the animal in the presampling position with high degree of accuracy each time (Supplemental Figure). This allowed us to measure plasma glucose concentration and FE throughout the study. The infusion protocol rapidly achieved the target plasma glucose [$>13.8 \text{ mmol/l}$ ($>250 \text{ mg/dl}$)], which was maintained for the duration of the experiment (Figure 2). The average steady-state plasma ^{13}C glucose FE was $56 \pm 4\%$. There were inter-animal variations in the reaching steady-state plasma FE (Figure 2a and 2b).

In vivo ^{13}C NMR spectra acquired during $[1,6\text{-}^{13}\text{C}_2]$ glucose infusion from a rat are shown in Figure 3. As observed in this figure, lipid signals at 30 ppm were absent, suggesting excellent localization. Incorporation of ^{13}C label into Glu and Gln was evident soon after the start of $[1,6\text{-}^{13}\text{C}_2]$ glucose infusion (Figure 3). Glu-C4, Glu-C3, Gln-C4 and Gln-C3 signal intensity progressively increased over time. The Glu-C4 linewidths in the last spectrum acquired prior to removal from the magnet and the first spectrum collected after repositioning were similar in each rat (Table 1), confirming that the procedure for blood collection did not impact B_0 shimming.

The Glu- $^{13}\text{C}_4$ and Glu- $^{13}\text{C}_3$ concentrations in the *in vivo* data were determined using the ^{13}C isotopic enrichment measured in the brain extracts using *ex vivo* MRS. The isotopic enrichments in Glu-C4 and Gln-C3 at the end of the study were $40 \pm 3\%$ and $27 \pm 2\%$, respectively. The time courses of ^{13}C label incorporation in Glu-C4 and Glu-C3 were fitted with the one compartment metabolic model (Figure 4). The V_{TCA} determined using this method was $0.67 \pm 0.13 \mu\text{mol/g/min}$.

Discussion

To our knowledge, this is the first study of oxidative glucose metabolism determined *in vivo* in the young rat brain using ^{13}C MRS. The V_{TCA} value obtained in the present study is comparable to the value determined using *ex vivo* methods in rats of comparable age [15] and supports the feasibility and reliability of our method for *in vivo* assessment of cerebral

oxidative metabolism in young rats. With additional studies, this approach may be extended to smaller rodents, such as neonatal rats and transgenic mouse models.

Multiple factors were critical for the successful application of *in vivo* ^{13}C MRS in this study. Glucose is an ideal substrate for understanding cerebral oxidative metabolism, since its transport across the blood brain barrier is not dependent upon plasma levels, except during severe hypoglycemia. Administration of glucose labeled in C1 and C6 positions improved sensitivity through greater enrichment in labeled metabolic products, since two molecules of $[3-^{13}\text{C}]$ pyruvate are produced per molecule of $[1,6-^{13}\text{C}_2]$ glucose [10,16]. Thus, we were able to achieve almost two-fold greater ^{13}C label incorporation in cerebral metabolites, compared with a previous *ex vivo* study that used $[1-^{13}\text{C}]$ glucose [13]. Additionally, the use of a high-field 9.4T magnet, optimal RF-coil design, efficient B_0 shimming and ^1H decoupling were all important factors contributing to high detection sensitivity [9,24] and ensured reliable detection of ^{13}C label in cerebral amino acids in the present study. Excellent three-dimensional localization eliminated any contamination from extracerebral lipid signals. This permitted direct detection at the ^{13}C frequency and avoided the need for ^1H -observed/ ^{13}C -edited MRS, which is more sensitive, but is limited by the significant overlap of Glu and Gln resonances [16].

An accurate knowledge of the time course of plasma glucose and isotopic enrichment (input function) is critical for deriving quantitative data from ^{13}C MRS using metabolic modeling [26]. A recent study demonstrated that metabolic modeling is possible without the knowledge of input function in large animals (non-human primates) [17]. However, a prerequisite for such analysis is rapid achievement of steady-state plasma FE that stays constant for the duration of the measurement [17]. As demonstrated in Figure 2, this may not be possible in small animals. Similarly, parallel bench-top experiments in littermates may help to infer the glycemia state in small rodents undergoing *in vivo* MRS [16]. However, the individual differences in FE are large enough that the input function needs to be measured for each animal individually. In this context, a key aspect of our study was the ability to collect blood samples from the young rats undergoing *in vivo* MRS, despite their low blood volume (approximately, 6–8 ml) [21]. The custom-designed animal holder permitted removal of the rats from the magnet for collecting small amount of blood (approximately, 1 ml for the entire experiment) for determining the input function. The high-degree of accuracy achieved while repositioning rats in the presampling location prevented significant degradation of B_0 shimming (Table 1). Thus, *in vivo* data could be acquired with minimal interruption and the entire ^{13}C labeling time course could be monitored in individual rats. Collectively, these technical and procedural refinements allowed us to achieve our goal using approximately 75% fewer animals than previous *ex vivo* studies in rats of comparable age [14,15].

Similar to our previous study in adult rats [24], the glucose infusion protocol rapidly achieved hyperglycemia. The approximately 55% isotopic enrichment of plasma glucose is higher than the approximately 40% enrichment achieved in previous studies of rats of comparable age [15,14] and slightly lower than the 62% enrichment achieved in adult rats [5]. Due to the broad chemical shift range of direct ^{13}C detection, we were able to detect and quantitate the time course of Glu-C4 and Glu-C3 resonances without contamination from Gln resonances in the brain. The approximately 44% enrichment of Glu-C4 is comparable to a previous report in 30-day-old rats [15]. The faster rate of ^{13}C labeling of Glu-C4, relative to Glu-C3, is similar to previous studies [15,14] and reflects labeling of the C4 position during the first turn of TCA cycle.

As we performed metabolic modeling using a one-compartment model, the V_{TCA} value in the present study primarily represents neuronal TCA cycle activity [10,28]. The V_{TCA} value

of 0.67 ± 0.13 $\mu\text{mol/g/min}$ in the present study is very similar to the combined (i.e. glutamatergic + GABAergic) neuronal V_{TCA} value of 0.71 ± 0.07 $\mu\text{mol/g/min}$ demonstrated using *ex vivo* ^{13}C MRS in 30-day-old rats [15]. The V_{TCA} value is also comparable to the values in the unstimulated adult rat brain [5,6,29]. This is not surprising, given that brain glucose metabolism approaches adult levels between 28 and 35 days of age in rats [2].

In summary, assessment of cerebral glucose oxidative metabolism using localized, direct-detected *in vivo* ^{13}C MRS is feasible in young rats. This methodology opens the avenue for evaluating brain energy metabolism during normal development and neuropathologies in small animals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Nehlig A. Respective roles of glucose and ketone bodies as substrates for cerebral energy metabolism in the suckling rat. *Dev Neurosci*. 1996; 18(5–6):426–433. [PubMed: 8940615]
2. Nehlig A. Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia. *Diabetes Metab*. 1997; 23(1):18–29. [PubMed: 9059763]
3. Vannucci RC, Vannucci SJ. Glucose metabolism in the developing brain. *Semin Perinatol*. 2000; 24(2):107–115. [PubMed: 10805166]
4. Gruetter R, Seaquest ER, Kim S, Ugurbil K. Localized *in vivo* ^{13}C -NMR of glutamate metabolism in the human brain: initial results at 4 tesla. *Dev Neurosci*. 1998; 20:380–388. [PubMed: 9778575]
5. Henry PG, Lebon V, Vaufrey F, Brouillet E, Hantraye P, Bloch G. Decreased TCA cycle rate in the rat brain after acute 3-NP treatment measured by *in vivo* ^1H - ^{13}C NMR spectroscopy. *J Neurochem*. 2002; 82(4):857–866. [PubMed: 12358791]
6. Hyder F, Rothman DL, Mason GF, Rangarajan A, Behar KL, Shulman RG. Oxidative glucose metabolism in rat brain during single forepaw stimulation: a spatially localized ^1H - ^{13}C nuclear magnetic resonance study. *J Cereb Blood Flow Metab*. 1997; 17(10):1040–1047. [PubMed: 9346428]
7. Mason GF, Gruetter R, Rothman DL, Behar KL, Shulman RG, Novotny EJ. Simultaneous determination of the rates of the TCA cycle, glucose utilization, alpha-ketoglutarate/glutamate exchange, and glutamine synthesis in human brain by NMR. *J Cereb Blood Flow Metab*. 1995; 15(1):12–25. [PubMed: 7798329]
8. Henry PG, Criego AB, Kumar A, Seaquist ER. Measurement of cerebral oxidative glucose consumption in patients with type 1 diabetes mellitus and hypoglycemia unawareness using (^{13}C) nuclear magnetic resonance spectroscopy. *Metabolism*. 2010; 59(1):100–106. [PubMed: 19766263]
9. Gruetter R, Adriany G, Choi IY, Henry PG, Lei H, Oz G. Localized *in vivo* ^{13}C NMR spectroscopy of the brain. *NMR Biomed*. 2000; 16(6–7):313–338. [PubMed: 14679498]
10. Henry PG, Adriany G, Deelchand D, Gruetter R, Marjanska M, Oz G, Seaquist ER, Shestov A, Ugurbil K. *In vivo* ^{13}C NMR spectroscopy and metabolic modeling in the brain: a practical perspective. *Magn Reson Imaging*. 2006; 24(4):527–539. [PubMed: 16677959]
11. Mason GF, Rothman DL. Basic principles of metabolic modeling of NMR (^{13}C) isotopic turnover to determine rates of brain metabolism *in vivo*. *Metab Eng*. 2004; 6(1):75–84. [PubMed: 14734257]

12. Scafidi S, O'Brien J, Hopkins I, Robertson C, Fiskum G, McKenna M. Delayed cerebral oxidative glucose metabolism after traumatic brain injury in young rats. *J Neurochem.* 2009; 109 Suppl 1:189–197. [PubMed: 19393027]
13. Pirttilä TR, Auriola SO, Kauppinen RA. Glucose metabolism in the developing cerebral cortex as detected by $^1\text{H}(^{13}\text{C})$ nuclear magnetic resonance spectroscopy ex vivo. *J Neurochem.* 1995; 64(1):417–423. [PubMed: 7798941]
14. Novotny EJ Jr, Ariyan C, Mason GF, O'Reilly J, Haddad GG, Behar KL. Differential increase in cerebral cortical glucose oxidative metabolism during rat postnatal development is greater in vivo than in vitro. *Brain Res.* 2001; 888(2):193–202. [PubMed: 11150475]
15. Chowdhury GM, Patel AB, Mason GF, Rothman DL, Behar KL. Glutamatergic and GABAergic neurotransmitter cycling and energy metabolism in rat cerebral cortex during postnatal development. *J Cereb Blood Flow Metab.* 2007; 27(12):1895–1907. [PubMed: 17440492]
16. Nabuurs CI, Klomp DW, Veltien A, Kan HE, Heerschap A. Localized sensitivity enhanced in vivo ^{13}C MRS to detect glucose metabolism in the mouse brain. *Magn Reson Med.* 2008; 59(3):626–630. [PubMed: 18224699]
17. Valette J, Boumezbeur F, Hantraye P, Lebon V. Simplified (^{13}C) metabolic modeling for simplified measurements of cerebral TCA cycle rate in vivo. *Magn Reson Med.* 2009; 62(6):1641–1645. [PubMed: 19780166]
18. Yamada KA, Rensing N, Izumi Y, De Erausquin GA, Gazit V, Dorsey DA, Herrera DG. Repetitive hypoglycemia in young rats impairs hippocampal long-term potentiation. *Pediatr Res.* 2004; 55(3):372–379. [PubMed: 14681492]
19. Kohn, DF.; Clifford, CB. Biology and disease of rats. In: Fox, JG.; Anderson, LC.; Loew, FM.; Quimby, FW., editors. *Laboratory animal medicine.* Second edn.. New York: Academic Press; 2002. p. 121-165.
20. Wood SL, Beyer BK, Cappon GD. Species comparison of postnatal CNS development: functional measures. *Birth Defects Res B Dev Reprod Toxicol.* 2003; 68(5):391–407. [PubMed: 14745989]
21. Lee HB, Blafox MD. Blood volume in the rat. *J Nucl Med.* 1985; 26(1):72–76. [PubMed: 3965655]
22. Rao R, Ennis K, Long JD, Ugurbil K, Gruetter R, Tkac I. Neurochemical changes in the developing rat hippocampus during prolonged hypoglycemia. *J Neurochem.* 2010; 114(3):728–738. [PubMed: 20477939]
23. Deelchand DK, Shestov AA, Koski DM, Ugurbil K, Henry PG. Acetate transport and utilization in the rat brain. *J Neurochem.* 2009; 109 Suppl 1:46–54. [PubMed: 19393008]
24. Deelchand DK, Ugurbil K, Henry PG. Investigating brain metabolism at high fields using localized ^{13}C NMR spectroscopy without ^1H decoupling. *Magn Reson Med.* 2006; 55(2):279–286. [PubMed: 16345037]
25. Gruetter R, Seaquist ER, Ugurbil K. A mathematical model of compartmentalized neurotransmitter metabolism in the human brain. *Am J Physiol Endocrinol Metab.* 2001; 281(1):E100–E112. [PubMed: 11404227]
26. Henry PG, Oz G, Provencher S, Gruetter R. Toward dynamic isotopomer analysis in the rat brain in vivo: automatic quantitation of ^{13}C NMR spectra using LCModel. *NMR Biomed.* 2003; 16(6–7):400–412. [PubMed: 14679502]
27. Tkac I, Rao R, Georgieff MK, Gruetter R. Developmental and regional changes in the neurochemical profile of the rat brain determined by in vivo ^1H NMR spectroscopy. *Magn Reson Med.* 2003; 50(1):24–32. [PubMed: 12815675]
28. Shestov AA, Valette J, Ugurbil K, Henry PG. On the reliability of (^{13}C) metabolic modeling with two-compartment neuronal-glial models. *J Neurosci Res.* 2007; 85(15):3294–3303. [PubMed: 17393498]
29. Hertz L, Dienel GA. Energy metabolism in the brain. *Int Rev Neurobiol.* 2002; 51:1–102. [PubMed: 12420357]

Figure 1.

Animal holder used for *in vivo* ^{13}C NMR spectroscopy in young rats. The animal holder and the mounting head function as a lock and key mechanism and guarantee fixed rotation angle. The inner ring of the mounting head facilitates fine adjustment of rotation (range: ± 20 degree) and the animal holder can be secured in this position for the duration of the study by tightening the two diagonally opposite rotation-tightening knobs. The translational (anterior-posterior) position can be adjusted using the fine adjustment knob on the holder while the translatable locking pin is anchored in the locking pin bracket on the mounting head. The optimal position is then secured using two translation-tightening knobs. Releasing the locking pin bracket and loosening the translation-tightening knobs enables removal of the holder from the magnet for blood draw. Returning the holder inside the magnet and locking the translation pin in the bracket guarantees repositioning of the rat in the exact presampling position.

Figure 2.

Plasma glucose concentration and ^{13}C fractional enrichment (FE) during $[1,6-^{13}\text{C}_2]$ glucose infusion in two 28-day-old rats. The ^{13}C glucose infusion was started at 0 min. Despite comparable plasma glucose concentrations (filled circles), the time taken to reach steady-state FE (open square) differed among the rats: While some rats achieved stable enrichment soon after the onset of infusion (**a**), steady-state FE was reached relatively late in others (**b**).

Figure 3.

The time course of ^{13}C label incorporation into glutamate (Glu) and glutamine (Gln) C4 and C3 resonances during $[1,6-^{13}\text{C}_2]$ glucose (Glc) infusion in a 28-day-old rat (TR = 2.5 s, NT = 256 for each ^{13}C spectrum). Inset shows the MRI in the coronal plane with the volume of interest from which the spectra were acquired (echo train length = 8, echo spacing = 15 ms, echo time = 48 ms, field of view = 2.5 cm $\times$ 2.5 cm, matrix = 256 $\times$ 256, slice thickness = 1 mm). * Refers to removal of the rat for blood draw.

Figure 4.

Glutamate C4 and C3 time courses and fits in a 28-day-old rat brain. The concentrations of glutamate (Glu) C4 (filled circles) and Glu-C3 (open squares) determined from the *in vivo* ^{13}C NMR data using LCMModel are plotted along the time axis. The continuous lines represent the best fits of the metabolic model to the time course data.

Table 1

Effect of repeated removal from the magnet for blood sampling and repositioning on glutamate-C4 (Glu-C4) linewidth in individual rats

Time after ¹³ C glucose infusion (min)	Relationship to removal/ repositioning in the magnet	Rat Number and Glu-C4 Linewidth (Hz)			
		1	2	3	4
59	Prior to removal	12.97	9.98	8.36	8.27
64	After repositioning	11.48	5.97	9.09	8.26
91	Prior to removal	13.20	6.82	9.52	9.48
96	After repositioning	10.13	7.39	10.30	8.86
128	Prior to removal	9.39	-	11.84	7.58
133	After repositioning	9.65	-	9.33	9.55

Each instance of removal from the magnet, collection of blood sample and repositioning of the holder inside the magnet took approximately 2 min.